Maturation and Fecundity of Brook Trout from Infertile Streams[1]

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ABSTRACT

The reproductive rate of brook trout populations from infertile Pennsylvania streams was determined. The standing crops for eight streams varied between 14 and 41 lb/acre. Trout in age-groups 0 through III were found to grow slowly with very few individuals reaching a total length of 6 inches.

The seasonal development of ova was determined by measuring all ova larger than 0.2 mm from selected females. The maturing ova of most females of age-groups II and III increased in diameter from 0.9 mm in February to 1.0 mm in June, 1.75 mm in July, 2.5 mm in August, 3.0 mm in September, and 4.0 mm in October. Maturing females could be distinguished from immature females by late August or early September on the basis of ova diameters. The mean diameter of ripe ova from 67 females was 4.05 mm with a range between 3.37 and 5.01 mm for individual trout ranging from 4.6 to 8.6 inches total length.

The relationship between the number of mature ova and the total length of the female for one population was described by a curvilinear regression ($\log Y = -0.5361 + 3.23 \log X$, where Y = number of eggs, and X = the total length of the fish) for brook trout between 3.6 inches (18 eggs) and 7.7 inches (213 eggs). Variation in this relationship existed among streams.

Ova production was estimated as 9050 ova per acre for one population and 13,620 ova per acre for another. Age-group-I females contributed approximately 9% of this production; age-group-II females, 54%; and age-group-III females, 37%. An ecological life table was constructed for two populations comparing rates of survival, age-specific fecundity rates, and reproductive rates.

INTRODUCTION

The brook trout, *Salvelinus fontinalis*, is widely distributed over eastern North America, often being found in small, infertile streams of the Appalachian Mountains. Trout from these streams grow very slowly although the trout populations are sparse. The populations are quite resilient, despite exploitation by anglers and even heavy reduction by fish toxicants (Cooper et al., 1962). It was considered desirable, therefore, to measure the reproductive ability of such populations from infertile streams and to compare these populations with previous reports from other parts of the species range (Vladykov, 1956; Rounsefell, 1957; and McFadden, 1961).

The 1961 collections of brook trout were obtained from streams in the northwestern part of Pennsylvania known as the Allegheny plateau, while the 1963 and 1964 collections were taken from the streams in the ridge-and-valley

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area in the central part of the state. In both areas the bedrock formations were sandstones and shales; the forest cover was second growth northern hardwoods with a few scattered conifers. Shrubs bordered a few of the streams.

The stream bottoms were composed of large stones, broken pieces of shale, gravel, and sand. Turbidity in these streams was low, even though the flow fluctuated greatly from spring to summer. The streams studied varied in width from 5 to 10 ft. Gradients varied from 110 to 520 ft/mile. Total alkalinities ranged from 12 to 38 ppm; the *p*H ranged from 6.8 to 7.5 (Table I). Streams of similar water quality have been shown to be quite unproductive of fish in this area (McFadden and Cooper, 1962). Aquatic plants and bottom organisms were scarce. Sixteen species of fishes were collected from these streams (Table II), although only the brook trout and sculpins (*Cottus bairdi* and *C. cognatus*) could be considered abundant. The brown trout (*Salmo trutta*), blacknose dace (*Rhinichthys atratulus*), longnose dace (*Rhinichthys cataractae*), and creek chub (*Semotilus atromaculatus*) were commonly collected; the remaining species were rare.

METHODS

Population Density

Estimates of the populations of brook trout were made by the mark-and-recapture method (Ricker, 1958). Confidence intervals were determined from

Table I. Physical and chemical characteristics of the Pennsylvania study streams. (Gradients were determined from U.S. Geological Survey maps. The mean width of the stream, total alkalinity, and *p*H were determined at low water levels for all streams.)

Stream	Length of fish sample area (*ft*)	Mean width (*ft*)	Gradient (*ft/mile*)	*p*H	Total alkalinity (*ppm*)
Long's Run	900	9	130	6.9	14
Whiting Run	1920	6	520	7.2	15
Dry Brook	2800	6	235	7.1	18
Rock Run	2200	5	125	6.9	15
No Name Run	2000	5	300	6.8	12
Dahoga Run	1400	6	170	6.8	12
Bobb's Creek	1000	5	185	7.1	17
Tomtit Run	2500	9	120	6.8	12
Lingle Stream	1950	9	150	7.5	16
Cherry Creek	2500	5	110	7.3	38
Crystal Run	960	6	255	6.9	18
Jaybuck Creek	–	5	200	7.3	15

TABLE II. Fishes associated with brook trout in selected streams. (Abbreviations of streams follow the order printed in Table I. Species present indicated by a plus sign.)

Species	Stream (abbreviated)											
	LR	WR	DB	RR	NNR	DR	BC	TR	LS	CC	CR	JC
Petromyzontidae												
Ichthyomyzon sp.										+		
Salmonidae												
Salmo gairdneri									+			+
Salmo trutta					+			+	+	+		
Cyprinidae												
Campostoma anomalum												+
Clinostomus elongatus											+	+
Rhinichthys atratulus	+					+	+		+	+		+
Rhinichthys cataractae	+						+			+		+
Semotilus atromaculatus		+		+			+		+	+		+
Semotilus margarita										+		
Catostomidae												
Catostomus commersoni	+					+				+		
Hypentelium nigricans										+		
Centrarchidae												
Lepomis gibbosus									+			
Percidae												
Etheostoma flabellare										+		
Cottidae												
Cottus bairdi		+	+	+	+	+	+			+	+	+
Cottus cognatus	+							+	+			

table 1.3.1 of Snedecor (1956). Fish were captured with electrofishing gear, were measured, marked with a fin-clip, and put back into the stream. On the following day, the stream section was treated with emulsified rotenone. The rotenone mixture was traced downstream with fluorescein dye and finally neutralized with potassium permanganate. All fish were killed; these were collected and preserved for analysis.

Each stream section was measured (Table I). The density of trout in each section was calculated from length–weight measurements and the population estimates. Population estimates were not made for the samples taken in February, March, April, and December; at water temperatures below 45 F, the behavior of the fish makes electrofishing very ineffective.

With the exceptions of Long's Run and Tomtit Run, each stream was sampled only once. However, the length of stream sampled (900–2500 ft) is believed to be adequate to assure a random sample of the brook trout populations. Population estimates indicated that more than 90% of the fish initially present in a sample area were recovered for study.

Age Composition and Growth

All fish collected with rotenone in 1961 were fixed in 10% formalin and later transferred to 40% isopropyl alcohol. Those collected in 1963 and 1964 were fixed and retained in 10% formalin. Total lengths and weights were taken from preserved fish.

Scale samples were taken for age determination from all trout whose age was in doubt. An analysis of size frequency combined with selected scale samples was adequate in delineating age-group 0. The scales were mounted, viewed with a microprojector, and the age determined from annuli. A description of the annuli and the validity of their use in age determination are given for this species by Cooper (1951).

Sex Ratios

Secondary sex characteristics such as the increased body depth, hooked lower jaws, and black coloration on parts of the head in the males (Vladykov, 1954) could be used in separating sexes externally among the larger trout immediately prior to the spawning season. However, the smaller fish did not show these characteristics. The sex of each fish was therefore determined by dissection and examination of the gonads, using a microscope when necessary.

In young-of-the-year and other small trout, the testes are transparent and rounded in cross-section, lying along the swimbladder. By contrast, in brook trout as small as 1.5 inches, the ovaries are flattened anteriorly, are whitish (less transparent than the testes), and contain ova which can easily be seen at a magnification of 25 diam. A detailed account of the morphology of the testes and ovaries of brook trout is given by Henderson (1962, 1963a).

The sex ratio for each population was determined for each age-group as a percentage of the females in the population. Confidence intervals at the 95% level were determined for each age group of the combined data following Simpson et al. (1960). Probability values of the hypothesis that the observed sex ratios were no different than a 1:1 ratio were determined for the larger samples using the adjusted chi-square distribution (Snedecor, 1956).

Maturation

Size and Age at First Maturity

The collections made in September and October immediately prior to spawning were used to determine the size and age of the trout at first maturity. Macroscopic examination of the gonads at other times cannot be relied upon to indicate whether or not a fish is mature, i.e. will spawn in that year. For these 2 months, the percentage of mature fish was calculated and compared by categories of size, age, and sex.

Maturity Index

The maturity index was calculated as the gonad weight $\times$ 100 divided by the body weight (Vladykov, 1956). The index was calculated for each fish in

age-groups I, II, and III. The mean maturity index for each age-group was then plotted by month to ascertain the seasonal development of the gonads.

SEASONAL MATURATION VS. EGG SIZE

Mature females were distinguished from immature females at different times of the year on a basis of egg size. A quantitative analysis of this observation was made as follows: Since similar distributions of egg size occurred in both ovaries of a female, the left ovary was arbitrarily selected to determine the mean diameter of the largest ova for each female. Ova under 2 mm in diameter were measured with an ocular micrometer. Ova larger than 2 mm in diameter were measured in a trough similar to that described by Lagler (1956) in a manner as outlined by Allen (1958). The mean diameter of the 30 ova was then compared with the total length of the fish for each of the monthly samples.

RECRUITMENT OF MATURE EGGS

Frequency distributions of the size of ova in individual brook trout were obtained for each age-group for each month of the year. This was done as follows: The female nearest to the mean length of the age-group for each month was selected. Both ovaries were dissected and each egg larger than 0.2 mm was measured along two perpendicular axes with an ocular micrometer. The method of averaging two measurements of each egg was shown to produce less variance in samples than did the practice of using one measurement (Higham and Nicholson, 1964). Measurements of eggs in both ovaries were then pooled in constructing the size–frequency diagrams.

SIZE OF RIPE EGGS

The size of ripe ova (those lying loose in the coelom) was compared with the size of individual females in the following manner: Samples of ripe female brook trout were obtained at weekly intervals during the fall of the year by electrofishing in Tomtit Run and were anesthetized with tricaine methanesulfonate (M.S. 222). Ripe ova were stripped from females directly into neutral buffered formalin to ensure against rupturing the ova (Hayes, 1949). Samples of 1–36 ripe ova (as available) were thus obtained from 67 females from one population.

FECUNDITY

DEFINITIONS

Fecundity is defined here as the total number of mature ova present in a female immediately prior to spawning. Since some of the maturing ova degenerate during development (Vladykov, 1956; Henderson, 1963b), fecundity was determined by direct count just prior to the spawning season and while the ovaries were still intact. All measurements of ova were taken from preserved material.

Three major classes of ova in brook trout are described by Vladykov (1956):

(1) *Recruitment stock*: Ova slightly less than 1 mm in diameter, yolkless, and transparent in the fresh condition, but becoming opaque in preserved material.

(2) *Maturing ova*: Ova varying from 1 to more than 4 mm in diameter, increasing in yolk content with growth.

(3) *Atretic ova*: Maturing ova which stop growing and gradually degenerate.

FECUNDITY RELATED TO FISH SIZE

Data relating the size of the fish to the number of mature eggs produced in both ovaries are restricted to females ranging in total length from 3.6 to 7.7 inches. This is the extreme lower limit of the range of mature brook trout. The samples are also restricted to those taken nearest to the spawning season in four streams. This restriction on time of collection is necessary because of the atresia of eggs in ovaries during development.

Regression analyses were done to obtain equations useful in predicting the number of eggs from fish length, fish weight, and ovary weight. Statistical methods follow Snedecor (1956) and Simpson et al. (1960).

RESULTS

Estimates of the Brook Trout Populations

Several vital statistics of the brook trout populations are necessary to interpret the results of maturation and fecundity presented later. In this section, therefore, brief summaries of population density, age composition, growth, length–weight relationship, and sex ratio are presented.

POPULATION DENSITY

The brook trout was the dominant species in these infertile streams with standing crops varying between 14 and 41 lb/acre (Table III). The number of trout in the different streams varied widely, taking into consideration the size of the sample areas. It must be remembered that these streams were originally selected as those containing a good population of brook trout. Not all streams in the area are like these.

AGE COMPOSITION AND GROWTH

No trout in their 5th year of life were found in any of the samples; trout in their 4th year (age-group III) were rare. Considering the fact that each stream was sampled in a different month, only minor differences in growth are apparent among the different populations. By the end of the growing season the mean length of different age-groups is approximately as follows: age-group 0, 3.0 inches; age-group I, 4.5 inches; age-group II, 5.5 inches; and age-group III, 6.5 inches (Table IV). Most of the brook trout in these infertile streams mature, spawn, and die before reaching 6 inches in total length.

TABLE III. Population estimates of brook trout from eight streams. (Estimates are based on the mark-and-recapture method. Confidence intervals were determined using table 1.3.1 of Snedecor (1956).)

Stream	Area (*acres*)	Population estimates		
		Number of fish	95% confidence interval	Standing crop (*lb/acre*)
Dry Brook	0.39	159	127–207	15
Rock Run	0.25	176	157–205	30
No Name Run	0.23	323	285–372	26
Dahoga Run	0.19	249	205–311	36
Bobb's Creek	0.21	222	197–259	41
Tomtit Run	0.52	953	820–1182	36
Lingle Stream	0.40	503	433–624	39
Cherry Creek	0.29	148	126–222	21
Crystal Run	0.13	126	111–149	14

Male brook trout were found to grow slightly faster than females, when comparisons are restricted to large samples such as Tomtit Run and Lingle Stream (Table V). Although differences between the sexes for age-groups I, II, and III can be demonstrated (not so for age-group 0), they are small and little bias would result in combining the sexes for growth analyses.

LENGTH–WEIGHT RELATIONSHIP

The length–weight relationship for brook trout was determined by the method of least squares separately for the two largest samples (Tomtit Run and Lingle Stream) with the assumption that the exponential equation ($W = aL^b$) adequately describes this relationship, where W = weight of the trout in grams, and L = total length of the trout in inches. The solution of this equation for 696 trout from Tomtit Run is: $W = 0.15\ L^{3.00}$; for 392 trout from Lingle Stream, the solution is: $W = 0.16\ L^{3.05}$. Combining the data of Tomtit Run with those of Lingle Stream, an estimate of the length–weight relationship for 1088 trout from infertile, central Pennsylvania streams is described as $W = 0.15\ L^{3.04}$.

SEX RATIO

The sex ratio of brook trout was very close to the 1:1 ratio when data for all 12 streams are combined (Table VI). Only age-group II showed a preponderance of females (58.2%; $p < 0.001$). And, when the two largest samples are analysed separately, an adjusted chi-square test does not substantiate the hypothesis that the observed sex ratios were different from a 1:1 ratio.

TABLE IV. Growth of brook trout (sexes combined) in total length (*inches*) and weight (g) from 12 streams. (Data under each heading include, in order: mean, standard error of the mean, and the number of individuals.)

Stream	Date of collection	Age Group 0 Length	0 Weight	I Length	I Weight	II Length	II Weight	III Length	III Weight
Long's Run	Feb. 10, 1964 Mar. 16, 1964[a]	–	–	3.18 (0.060) 58	5.8 (0.27) 58	5.46 (0.167) 31	29.6 (2.78) 31	6.42 (0.294) 4	50.5 (9.94) 4
Whiting Run	Apr. 11, 1961	–	–	3.28 (0.066) 42	5.3 (0.38) 42	4.71 (0.173) 14	16.6 (1.18) 14	6.28 (0.260) 6	38.6 (4.59) 6
Dry Brook	May 16, 1961	–	–	3.75 (0.063) 71	9.3 (0.46) 71	5.01 (0.084) 25	21.1 (1.14) 25	6.44 (0.301) 5	47.7 (8.22) 5
Rock Run	June 7, 1961	1.29 (0.026) 9	0.4 (0.01) 9	3.75 (0.066) 80	10.0 (0.55) 80	5.24 (0.053) 73	25.6 (0.90) 73	6.04 (0.085) 7	39.2 (1.74)
No Name Run	July 10, 1961	2.32 (0.031) 120	2.2 (0.10) 120	4.24 (0.075) 49	13.7 (0.82) 49	5.19 (0.083) 52	24.9 (1.43) 52	–	–
Dahoga Run	Aug. 11, 1961	2.45 (0.124) 33	2.3 (0.11) 33	4.05 (0.444) 115	11.4 (4.24) 115	5.26 (0.098) 17	27.1 (1.96) 17	5.97 (–) 3	40.7 (–) 3
Bobb's Creek	Sept. 11, 1961	2.53 (0.025) 93	2.5 (0.09) 93	4.18 (0.052) 80	11.9 (0.51) 80	5.55 (0.179) 11	29.6 (3.21) 11	–	–
Tomtit Run	Sept. 20, 1963	2.54 (0.015) 250	2.6 (0.05) 250	3.96 (0.021) 305	9.7 (0.16) 305	5.20 (0.045) 111	22.3 (0.72) 111	6.15 (0.153) 30	40.3 (3.57) 30
Lingle Stream	Oct. 5, 1964	2.89 (0.023) 159	4.1 (0.09) 159	4.33 (0.030) 112	13.5 (0.21) 112	5.58 (0.064) 92	31.1 (1.05) 92	6.77 (0.103) 28	56.0 (2.54) 28
Cherry Creek	Oct. 6, 1961	3.02 (0.040) 52	4.4 (0.18) 52	4.70 (0.073) 63	17.2 (0.85) 63	5.48 (0.112) 15	27.6 (0.22) 15	7.25 (–) 2	62.9 (–) 2
Crystal Run	Nov. 10, 1961	2.48 (0.036) 60	2.4 (0.13) 60	4.00 (0.120) 16	10.0 (0.96) 16	4.71 (0.117) 16	15.8 (1.10) 26	5.90 (–) 1	32.4 (–) 1
Jaybuck Creek	Dec. 5, 1961	2.78 (0.180) 29	3.2 (0.22) 29	4.25 (0.056) 46	11.8 (0.50) 46	5.28 (0.081) 8	20.8 (1.15) 8	8.30 (–) 1	49.8 (–) 1

[a]Collections for Feb. 10, 1964, and Mar. 16, 1964, combined.

TABLE V. Comparison of growth in total length between male and female brook trout from two streams.

Age-group	Males: Number of individuals	Males: Mean length (*inches*)	Males: Standard error of mean	Females: Number of individuals	Females: Mean length (*inches*)	Females: Standard error of mean	*p*
Tomtit Run							
0	134	2.53	0.019	116	2.54	0.014	>0.70
I	146	4.04	0.031	159	3.90	0.028	<0.001
II	51	5.33	0.067	60	5.09	0.058	<0.01
III	12	6.43	0.300	18	5.96	0.170	<0.001
Lingle Stream							
0	78	2.89	0.035	81	2.89	0.032	>0.95
I	60	4.43	0.061	52	4.21	0.056	<0.01
II	43	5.87	0.102	49	5.32	0.061	<0.001
III	14	6.96	0.137	14	6.59	0.143	<0.10

TABLE VI. Sex ratios of brook trout from 12 streams given as the percentage of females. (Confidence intervals at the 95% level are given in parentheses. Probability values on the hypothesis that the observed sex ratios were no different from the theoretical 1:1 ratio were based on the adjusted chi-square distribution following Snedecor (1956, p. 218).)

Stream	Age Group 0: N	0: %F	I: N	I: %F	II: N	II: %F	III: N	III: %F
Long's Run	–		58	38.7	31	61.3	4	50.0
Whiting Run	–		42	42.9	14	71.4	6	33.3
Dry Brook	–		71	53.5	25	56.0	5	80.0
Rock Run	–		80	57.5	73	57.5	7	57.1
No Name Run	120	42.5	49	49.0	20	80.0	–	
Dahoga Run	33	57.6	115	53.9	17	48.8	3	66.7
Bobb's Creek	93	45.2	80	43.8	11	63.6	–	
Tomtit Run	250	46.4	305	52.1	111	54.1	30	60.0
Lingle Stream	159	50.9	112	46.4	92	53.3	28	50.0
Cherry Creek	52	59.6	63	49.2	15	53.3	2	50.0
Crystal Run	60	50.0	16	75.0	16	62.5	1	00.0
Jaybuck Run	29	58.6	46	60.9	8	87.5	1	00.0
All Streams:	796	48.6 (45.1–52.1) $p>0.30$	1035	50.8 (47.7–53.9) $p>0.50$	433	58.2 (52.9–63.5) $p<0.001$	87	54.0 (43.5–64.5) $p>0.50$

MATURATION

AGE AT FIRST MATURITY

Sexual maturity was attained by the majority of the brook trout at the end of their 3rd year of life, although many males and females were mature in 2 years, and one male was mature at the end of its 1st year. Size also influenced sexual maturity in these populations. This is best demonstrated in age-group II where the percentage maturity of both males and females increases with increase in total length (Table VII). For both age and size, there is wide variability in the time of first sexual maturity. One male was mature at 1 year, 3.4 inches long; yet a 4-year-old male 6.7 inches long was still immature. Two females were mature at 2 years, 3.6 inches long; yet a 3-year-old female was still immature at 5.8 inches.

SEASONAL CHANGES IN MATURITY INDEX

The annual development of the gonads can be traced by calculating the mean maturity index for fish collected at different times of the year. Development begins in June for both sexes and reaches a peak in September (Fig. 1 and 2). There is some evidence that males mature earlier in the season than the females do, and that older (or larger) fish mature earlier in the season than do those spawning for the first time as 2-year-olds.

At the peak of sexual development, the testes amount to about 1.5% of the body weight of the fish. In the female this value for the ovary is about 10.0% of the body weight. In young fish a smaller proportion of their body weight goes into the seasonal egg or sperm production than it does in older fish.

SEASONAL MATURATION VS. EGG SIZE

Some females that would be mature in October can be distinguished from immature females as early as August on the basis of examining the mean diameter of 30 of the largest ova in the female (Fig. 3 and 4). Although the maturing ova increase rapidly in diameter in July, the ova of the larger recruitment stock cannot be distinguished with certainty from the smaller maturing eggs. By late August or early September a distinct separation in ova diameters occurs. However, a small number of females collected as late as August may resorb the entire lot of maturing ova before the spawning season. Three such females (of 80 examined) were noticed during this study; a 5.1-inch, age-group-I female collected from Bobb's Creek and two 4.9-inch, age-group-II females collected from Tomtit Run.

SIZE OF RIPE EGGS

The mean diameter of ripe ova from 67 brook trout ranging in length from 4.6 to 8.5 inches was 4.05 mm; ripe eggs from individual females varied in mean diameter from 3.35 mm to 5.0 mm. There was little correlation between the size of ripe ova and the age of the females. Females of similar size but of different age groups had ova of approximately the same diameter. However, there was positive correlation (coefficient of 0.645) between size of the ripe ova and the

TABLE VII. The relationship of size and age to sexual maturity in brook trout from infertile streams. (Total number of individuals (T); number of mature fish (M).)

Age-group	Total length (*inches*)	Bobb's Creek		Tomtit Run		Lingle Stream		Cherry Creek		Total streams	
		T	M	T	M	T	M	T	M	T	%M
					Males						
0	2.0–2.9	47	0	131	0	43	0	7	0	228	0.0
	3.0–3.9	4	0	3	0	35	1	14	0	56	1.8
	Total:	51	0	134	0	78	1	21	0	284	0.4
I	3.0–3.9	12	1	65	10	9	0	2	1	88	13.6
	4.0–4.9	29	18	78	18	42	12	15	6	164	32.9
	5.0–5.9	4	4	3	3	9	2	15	11	31	64.5
	Total:	45	23	146	31	60	14	32	18	283	30.4
II	4.0–4.9	–	–	11	0	4	2	–	–	15	13.3
	5.0–5.9	4	3	36	25	20	15	5	5	65	73.8
	6.0–6.9	–	–	3	3	14	12	2	2	19	89.5
	7.0–7.9	–	–	1	1	5	5	–	–	6	100.0
	Total:	4	3	51	29	43	34	7	7	105	69.5
III	5.0–5.9	–	–	4	4	–	–	–	–	4	100.0
	6.0–6.9	–	–	5	5	7	6	–	–	12	91.7
	7.0–7.9	–	–	2	2	7	7	1	1	10	100.0
	8.0–8.9	–	–	1	1	–	–	–	–	1	100.0
	Total:			12	12	14	13	1	1	27	96.3
					Females						
0	1.0–1.9	–	–	2	0	–	–	–	–	2	0.0
	2.0–2.9	42	0	111	0	43	0	16	0	212	0.0
	3.0–3.9	–	–	3	0	38	0	15	0	56	0.0
	Total:	42	0	116	0	81	0	31	0	270	0.0
I	3.0–3.9	16	3	103	0	13	0	2	0	134	2.2
	4.0–4.9	17	8	55	9	36	6	26	15	134	28.4
	5.0–5.9	2	1	1	0	3	0	3	3	9	44.4
	Total:	35	12	159	9	52	6	31	18	277	17.2
II	4.0–4.9	1	1	26	11	11	5	1	1	39	46.2
	5.0–5.9	3	3	31	23	36	29	7	5	77	77.9
	6.0–6.9	3	3	3	3	2	2	–	–	8	100.0
	Total:	7	7	60	37	49	36	8	6	124	69.4
III	4.0–4.9	–	–	1	1	–	–	–	–	1	100.0
	5.0–5.9	–	–	9	9	1	1	–	–	10	100.0
	6.0–6.9	–	–	6	6	10	10	1	1	17	100.0
	7.0–7.9	–	–	2	2	3	3	–	–	5	100.0
	Total:			18	18	14	14	1	1	33	100.0

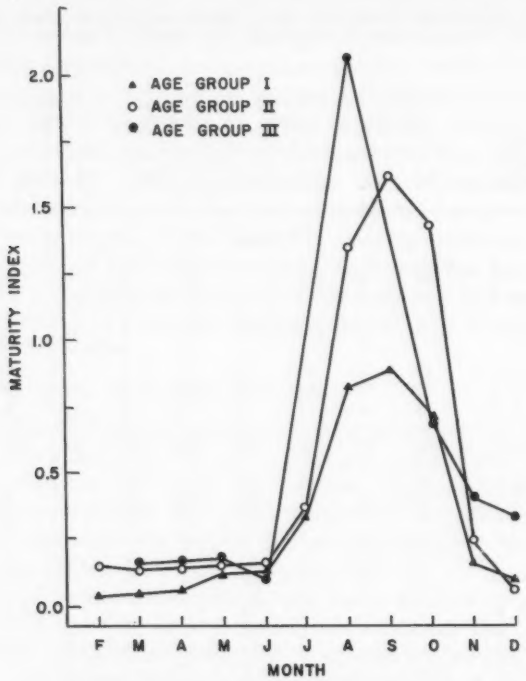

FIG. 1. The annual reproductive cycle of male brook trout as indicated by the mean maturity index (gonad weight × 100 divided by the body weight). Figure based on data from all streams.

total length of the females. A straight-line regression was determined as $Y = 2.71 + 0.23\ X$, where Y = mean diameter of the ripe ova in millimeters and X = total length of the female in inches (Fig. 5).

RECRUITMENT OF MATURE EGGS

Individual females of age-group II were chosen to illustrate the recruitment of mature ova during the annual reproductive cycle because samples of this age-group were available for all months. Females of other age-groups show similar cycles of recruitment. Little development of ova occurs before June in the annual cycle and graphs of several months are therefore omitted (Fig. 6). The largest ova in June are approximately 1.0 mm in diameter. These maturing ova grow rapidly to approximately 1.75 mm in July, 2.5 mm in August, 3.0 mm in September, and 4.0 mm in October. Although the maturing ova at this time make up most of the volume of the ovary, they are still only a small fraction of the number of eggs present.

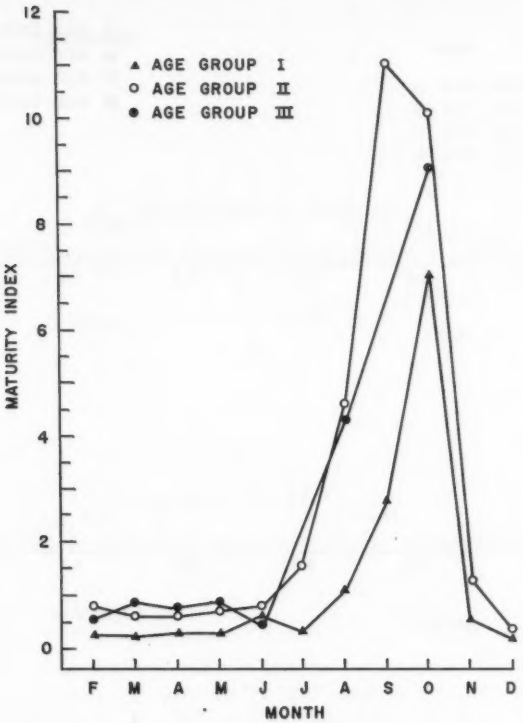

FIG. 2. The annual reproductive cycle of female brook trout as indicated by the mean maturity index (gonad weight × 100 divided by the body weight). Figure based on data from all streams.

DURATION OF SPAWNING SEASON

The extensive collecting done on Tomtit Run provided an estimate of the duration of the spawning season for brook trout in this area. Spawning begins late in September and extends into November (Fig. 7), as indicated by the distribution of 67 ripe females taken in six weekly samples of about 150 trout each in 1964. In 1963, a sample of 63 mature females collected by rotenone on September 20 did not contain any ripe fish. Weekly collecting to determine the extent of spawning in 1964 was terminated on October 28 because of the potential interference with the hunting season for wild turkey, although it appears likely that the majority of spawning had occurred by that date. Only a few unspawned females were still present in the area when collecting was terminated.

Large females in a population tend to spawn earlier than smaller females do. Measurements of the 67 ripe females examined in Tomtit Run during the

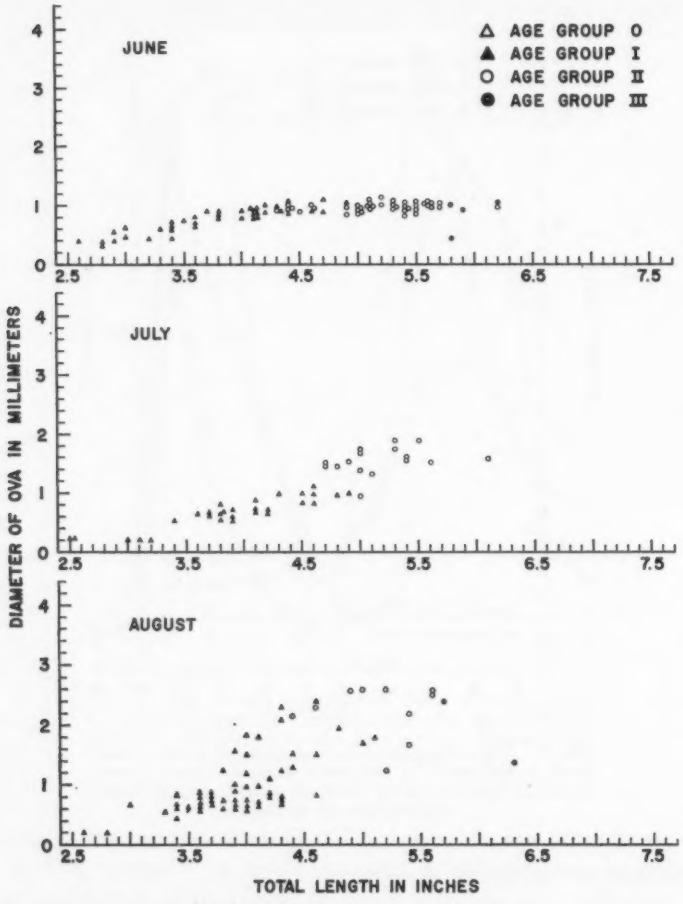

FIG. 3. The separation of maturing females from immature females on the basis of ova diameter for June, July, and August. (Each point is the mean of 30 largest ova from the left ovary of individual females.)

period September 22–November 4, 1964, were partitioned into three groups and the mean lengths calculated. Mean lengths decreased uniformly over the spawning season as follows:

Date	*Number of females*	*Mean length of spawning females*
September 22, October 14	23	6.03 inches
October 21	26	5.87 inches
October 28, November 4	18	5.57 inches

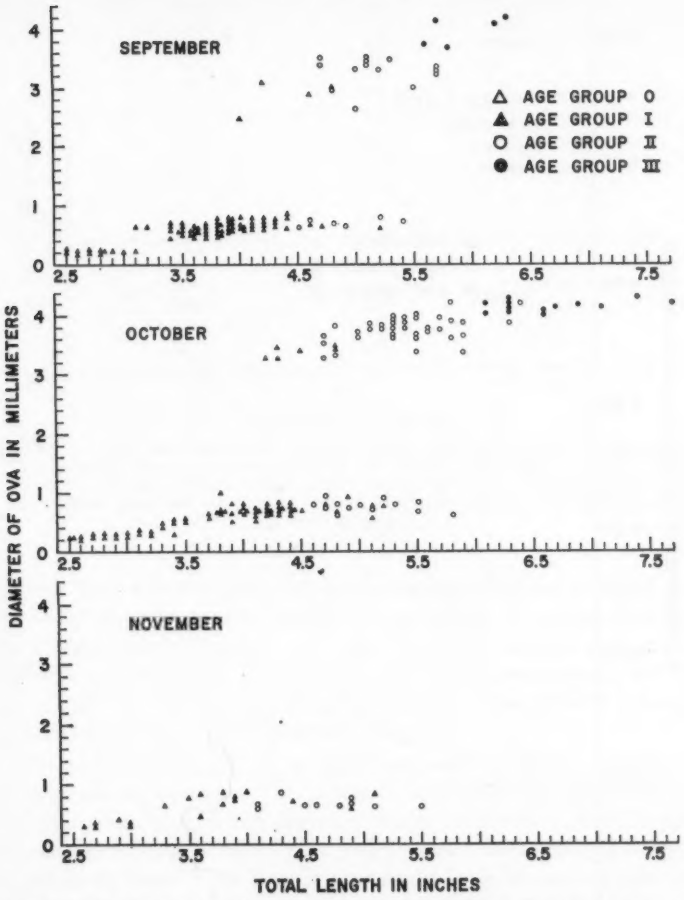

FIG. 4. The separation of maturing females from immature females on the basis of ova diameters for September, October, and November. (Each point is the mean of 30 largest ova from the left ovary of individual females.)

The difference between the mean lengths for the first and third groups approaches significance ($0.10 > p > 0.05$).

There is some evidence that the trout spawn in other study streams at about the same time as in Tomtit Run. In Lingle Stream, 2 of 56 maturing females were ripe on October 5, 1964; in Cherry Creek, 8 of 25 maturing females were ripe on October 7, 1961.

FECUNDITY

The distribution of the number of large maturing ova (>2.4 mm in diameter) among the left and right ovaries was often unequal, although there

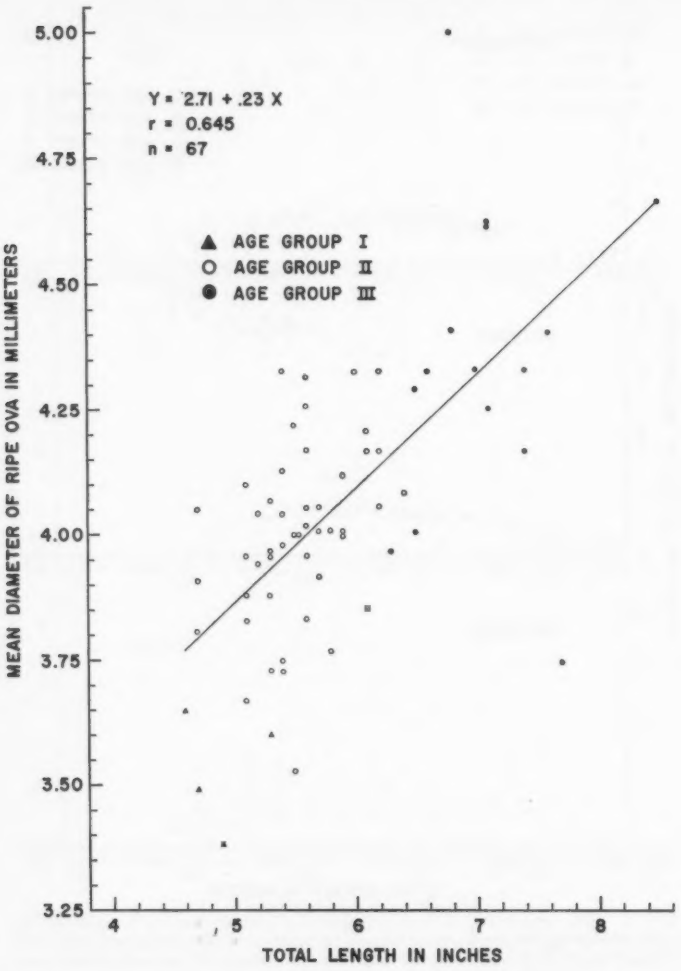

FIG. 5. Variation in the sizes of ripe ova from brook trout from Tomtit Run. (The regression line was calculated by the method of least squares.)

was no consistent bias toward a greater number in either ovary. In the combined data for four streams (151 females) 68 had a larger left ovary, 76 had a larger right ovary, and 7 had ovaries of approximately equal size (Fig. 8). Chi-square tests (Snedecor, 1956, p. 218) did not substantiate the hypothesis that differences in egg number existed between left and right ovaries. It was considered desirable, therefore, to use the total number of maturing ova in a female to explore the relationship between fecundity and size of the fish.

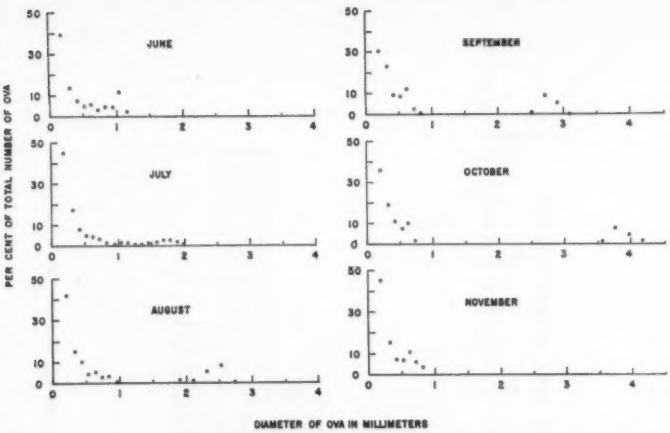

FIG. 6. Size distribution of ova from individual brook trout of age-group II showing the development during the annual reproductive cycle. (The number of eggs in both ovaries of a representative fish for each month are as follows: June, 480; July, 1193; August, 1081; September, 1573; October, 516; November, 643.)

Tomtit Run was selected to examine this relationship in detail because of the relatively large sample available (63 females). A scatter diagram of the number of eggs plotted against the total length of the fish indicated that an exponential equation would describe this relationship adequately, with the mean number of eggs per female increasing from about 18 eggs for a 3.6-inch female to about 213 eggs for a 7.7-inch female (Fig. 9).

Three additional populations were analysed to detect differences in this relationship among streams. This analysis is confounded somewhat by differences in time of collection and therefore stage of maturity, but it appears that trout populations from Bobb's Creek and Cherry Creek produce nearly twice as many eggs as do those from Tomtit Run and Lingle Stream (Table VIII). The high production of eggs as indicated in the Bobb's Creek sample would be reduced slightly by atresia before spawning, but this bias would not operate in the Cherry Creek population.

The number of mature ova were also compared to the weight of the female. This relationship can best be described by a straight line with the number of eggs increasing from about 30 for a 13-g female to about 200 for a 70-g female (Fig. 10). A similar relationship exists between the number of mature ova and the total weight of the ovaries (Fig. 11).

DISCUSSION

MATURATION

The brook trout as a species is inherently capable of maturing and spawning at the end of its 1st year of life. This generalization is the one best supported

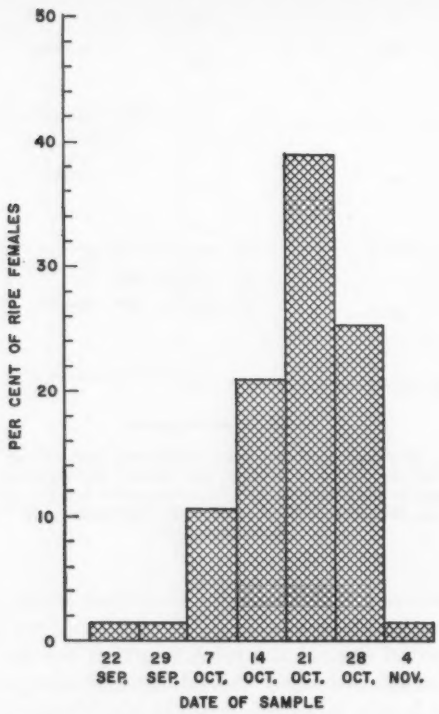

FIG. 7. The spawning season of brook trout from Tomtit Run in central Pennsylvania. (The *y*-axis represents the percentage of 67 ripe females that were examined during the fall of 1964.)

by all the evidence available. However, the time of maturation may be affected by many factors, perhaps including inheritance.

A slow rate of growth (less than 3.5 inches for the first year, or less than 4.5 inches for 2 years) may seriously retard maturation. When this growth rate is exceeded in wild populations, brook trout may commonly mature at 2 years of age (McFadden, 1961). Or, under some fish-cultural regimes, both sexes may mature at the end of their 1st year (Seguin, 1951).

Poor nutrition at the season of the year when egg development is normally rapid may also result in complete lack of development. In the latter case, such a fish would be judged mistakenly as immature even though it had spawned in the previous year. Alm (1959) gives an explicit account of maturation in brown trout in which he distinguishes between a mature fish (inherently capable of spawning in that year) and a ripening fish (developing mature eggs in that year). It is difficult, however, in his paper to ascertain how he identifies such a mature, but unripe individual.

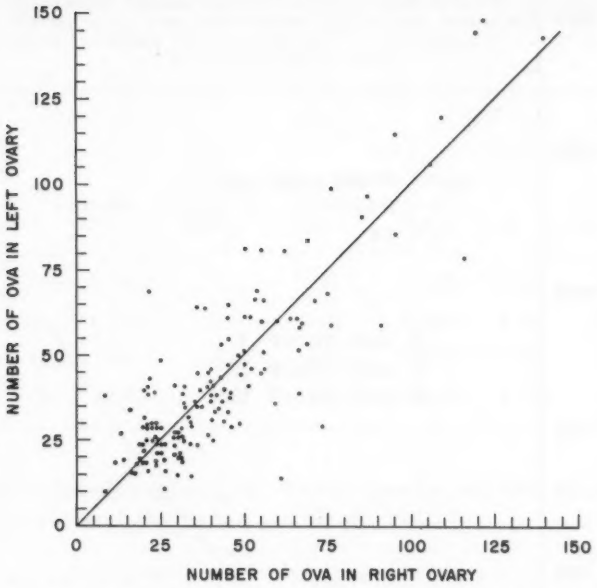

FIG. 8. A comparison of the number of mature ova in the left ovary with the number of mature ova in the right ovary from 151 brook trout. (The diagonal line indicates equal numbers of mature ova in both ovaries. Data combined from September and October collections from four streams.)

Mistakes in identification of mature, unripe fish as immature ones could partially account for the apparent late maturation of fast-growing brook trout populations in Maine (Cooper and Fuller, 1945), and in Manitoba (Doan, 1948), but such delayed maturation cannot be due entirely to poor nutrition or failure to spawn each year following maturation. In both of these populations, many fish longer than 14 inches were judged to be immature because of the lack of egg development.

Delayed maturation in some populations of brook trout may be an inheritable characteristic although no critical evaluation of this hypothesis has yet been attempted. Genetic control of maturation has been reported for the brown trout (*Salmo trutta*) by Alm (1949), and for the Arctic char (*Salvelinus alpinus*) by Maar (1949).

ATRESIA

Only a small percentage of the recruitment stock of eggs eventually mature and are spawned (Fig. 6). Under poor conditions of nutrition and growth, all eggs may stop development and be resorbed. When conditions are favorable for growth, such as in hatcheries, as little as 5% of the developing eggs may

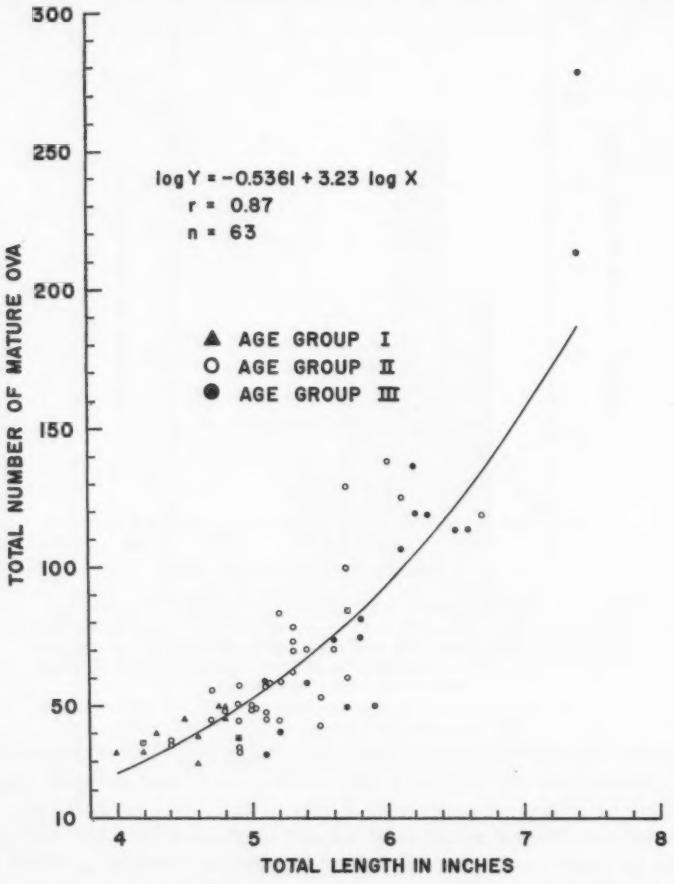

FIG. 9. Regression of the number of mature ova compared with total length for brook trout from Tomtit Run in central Pennsylvania.

become atretic (Henderson, 1963b), the continuing growth of the fish making room for the increase in size of the individual eggs. In most wild populations, however, losses to atresia are probably greater than 50% (Vladykov, 1956), and in the very small mature females in the present study the rate of atresia is probably higher than 50% (Fig. 6). The environment thus exercises a large measure of control over egg production through the nutrition of the ovary and growth of the female (Woodhead, 1960). This has been demonstrated experimentally for the rainbow trout (*Salmo gairdneri*) by Scott (1962).

Retention of ripe eggs in the coelom of brook trout is slight under natural conditions in which spawning is possible. Only a few unspawned eggs were

TABLE VIII. Relationship between fecundity and total length of brook trout from four stream populations in Pennsylvania. (Ova from mature females with ovaries still intact. Values for constants determined empirically from relationship $Y = aX^b$, where Y = number of ova, and X = total length of female.)

Stream	Collection date	Number of females	Mean ovum diameter (*mm*)	(*log*) *a*	*b*	Calculated number of eggs for female of: 4 inches	7 inches
Bobb's Creek	Sept. 12, 1961	17	2.89	0.1570	2.68	59	264
Tomtit Run	Sept. 21, 1963	63	3.60	−0.5361	3.23	26	156
Lingle Stream	Oct. 5, 1964	54	3.82	−0.2757	2.96	32	168
Cherry Creek	Oct. 7, 1961	17	3.64	−0.1968	3.14	49	286

found in the November collections of spent females and this result has been reported by other workers (Brown and Kamp, 1942).

VARIATION IN FECUNDITY

Variability in egg production by different populations of brook trout is high, even after allowance is made for differences in the size of the females (Fig. 12). Such variability also exists among the populations in Pennsylvania (Table VIII). This is a common phenomenon among other salmonids (Rounsefell, 1957) and may be due to several different factors. An interesting theoretical explanation for differences in fecundity is given by Svardson (1949) in terms of natural selection by environmental factors. Under conditions of low intraspecific competition for food and space, there would be selection toward females with an inherent capacity to produce larger numbers of eggs. Heavy exploitation of the adult population and a resultant low total production of eggs would serve to maintain selection toward an increased fecundity.

To the contrary, if egg production of a population were high, a high intraspecific competition among the fry would result. Under these conditions, selection would be toward the production of a lesser number of eggs of greater size because of the competitive advantages for survival of fry coming from large eggs. Such a selection pressure would be maintained only so long as the number of eggs produced by the population did not fall below the number necessary to utilize all the individual territories available to the small fish (as indicated by Kalleberg, 1958; LeCren, 1962).

Despite the plausibility of such an explanation, the reported statistics of any natural brook trout population have been inadequate to explain differences in fecundity on a basis of natural selection. The high density of adult trout in Tomtit Run and Lingle Stream is in accord with the reduced fecundity (Table

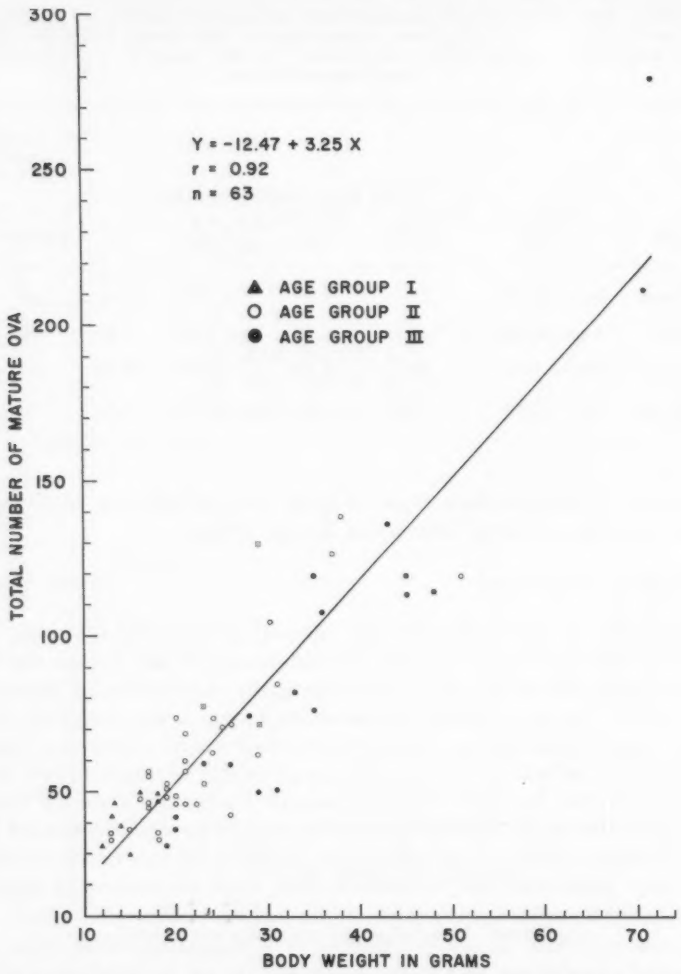

FIG. 10. Regression of the number of mature ova compared with body weight for brook trout from Tomtit Run in central Pennsylvania.

VIII). However, there is no distinctive evidence among our data that this trait is either genetically-determined or caused by environmental factors.

OVA PRODUCTION OF POPULATIONS

Salmonids produce a small number of large eggs when compared with most other fishes, and survival to hatching is usually higher. This has led investigators to use the egg production of a population as a rather accurate measure

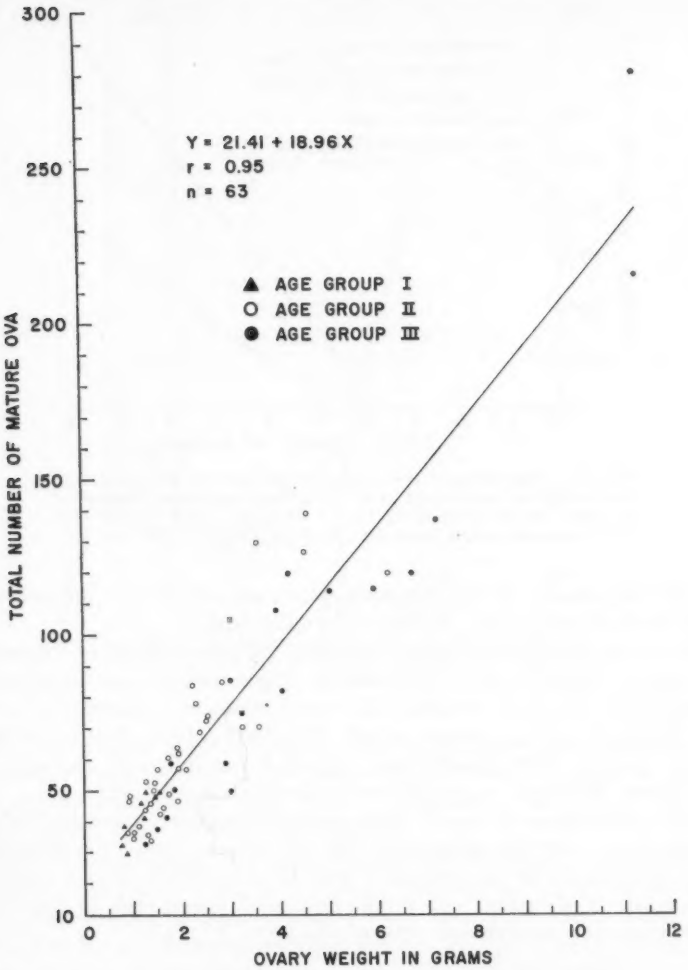

FIG. 11. Regression of the number of mature ova compared with ovary weight for brook trout from Tomtit Run in central Pennsylvania.

of the number of young with which a particular year-class originates. The difficulty of censusing small fry also has influenced the acceptance of this practice.

The density of ova production of a nonmigratory trout population may be derived from a schedule of several population parameters including, among others: (1) size of females, (2) percentage maturity of size- or age-groups of females, and (3) estimates of population density of females. For this reason,

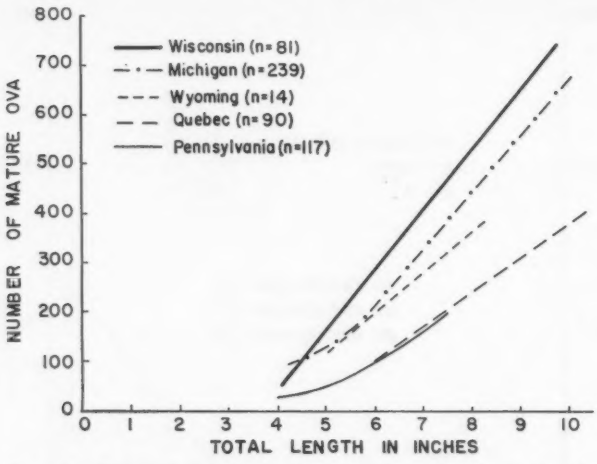

FIG. 12. Regression of the number of mature ova compared with total length for brook trout from five different localities. (Pennsylvania data are for trout from Tomtit Run and Lingle Stream; the other data are taken from fig. 11 of McFadden (1961, p. 35).)

there are few reports in the literature which are useful for comparison with the two streams intensively studied in Pennsylvania.

The production of brook trout eggs from two populations in Pennsylvania (9050/acre in Tomtit Run; 13,620/acre in Lingle Stream) are quite low when compared with the range of values (28,300–82,500/acre) reported for Lawrence Creek, Wisconsin, over a 4-year period (McFadden, 1961), but are within the range of values (2200–26,800/acre) reported for different sections of the Pigeon River, Michigan, over a 3-year period by Cooper (1953).

Most populations of brook trout studied intensively have had relatively short life spans, and the majority of the ova production of the population is usually contributed by age-groups I and II. The Pennsylvania populations show a delay in abundant egg production until age-groups II and III as a result of both slow growth and delayed maturity (Fig. 13).

REPRODUCTIVE RATE OF POPULATIONS

The reproductive ability of a population has often been described in the form of a life table depicting age-group composition, age-specific fecundity rate, and survival rate (Andrewartha and Birch, 1954). In the present investigation we are assuming that the two populations of brook trout are essentially stable; this assumption is a fair one based on age-composition of the stock and similarities to other populations studied. Our use of life tables is thus restricted to determining the interrelationships among the statistics given above, and in comparing the differences between populations. They cannot be used to determine whether or not the populations are changing from one year to the next.

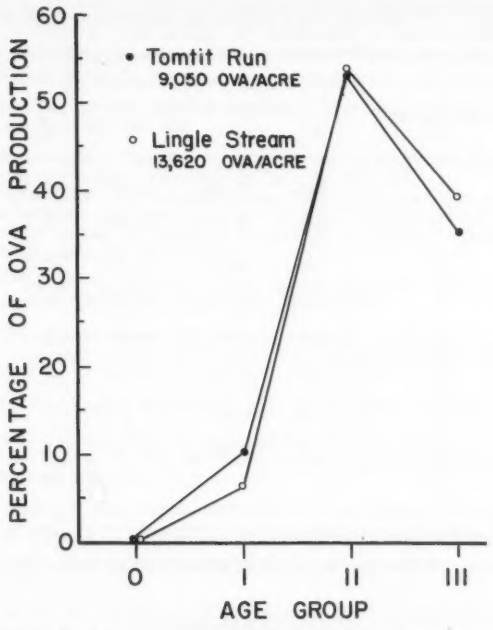

FIG. 13. The percentage of total ova production contributed by different age-groups of brook trout from two streams.

A similar analysis, conducted on five brown trout populations in Pennsylvania by McFadden and Cooper (1964) had indicated that populations remaining essentially in equilibrium with their environment, do so by concurrent variation among several of these parameters.

High ova production in Lingle Stream (13,620/acre) is accompanied by a low annual survival for the first 2 years, followed by higher survival in the last 2 years of life. In Tomtit Run a lower ova production (9050/acre) is accompanied by an increased survival during the first 2 years followed by a marked decrease in survival in the 3rd year. The net result is a similar number of mature fish in the two populations with similar reproductive rates to maintain the population equilibrium (Table IX).

The difference in survival between the two populations in the 1st year indicates the high degree of control exercised by the environment over the production of young fish, perhaps through territorial behavior (Kalleberg, 1958; LeCren, 1962). A limitation on increase in growth of brook trout in these small streams may also be due in part to feeding behavior (Cooper et al., 1962). The demonstrated effect of nutrition and size of the fish on maturity and fecundity are also measures of environmental control over reproduction of populations. When all of these interrelationships are considered, it is easy

TABLE IX. Ecological life table of two brook trout populations in Pennsylvania. For each year of life are given: number of individuals (p), annual survival rate (s), survival from birth to year x (1_x), age-specific fecundity rate (m_x), and reproductive rate ($1_x m_x$).

Stream	Age in years (x)	p	s	1_x	m_x	$1_x m_x$	$\Sigma 1_x m_x$
Tomtit Run	1	414	0.089	0.089	0.0	0.0	
	2	387	0.935	0.083	1.3	0.108	
	3	121	0.313	0.026	20.0	0.518	
	4	30	0.248	0.006	49.3	0.316	0.942
Lingle Stream	1	236	0.043	0.043	0.0	0.0	
	2	139	0.589	0.025	3.3	0.084	
	3	96	0.691	0.018	28.5	0.499	
	4	28	0.292	0.005	74.7	0.381	0.964

to be impressed with the variety of mechanisms available to either increase or decrease the size of the trout populations in these infertile streams.

ACKNOWLEDGMENTS

We are indebted to J. A. Boccardy for the brook trout collections made in 1961 as a part of a survey of the Allegheny National Forest. Field assistance for other collections was provided by Dr R. L. Butler, G. E. Krantz, C. D. Meyers, K. G. Miller, D. E. Samuel, W. M. Samuel, R. C. MacWatters, and R. C. Scherer. Help in designing a computer program for many of the calculations was given by S. J. Kasselman. Dr K. R. Bennett advised us on many aspects of statistical analysis.

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Sexual Maturity and Spawning of the American Plaice, *Hippoglossoides platessoides* (Fabricius), from Newfoundland and Grand Bank Areas[1]

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ABSTRACT

The peak spawning period of American plaice was the first part of April for Flemish Cap and ranged from the end of April for the Grand Bank and surrounding areas to the first part of June for Labrador–Northeast Newfoundland Shelf. Average bottom temperatures during spawning ranged from about 3.5 C for Flemish Cap and the deep spawning areas to the north, to about –1.3 C for St. Mary's Bay and the northern half of the Grand Bank. For the Southeast Grand Bank, Southwest Grand Bank, and St. Pierre Bank average spawning temperatures were 1.1, 2.8, and 2.7 C, respectively.

There was evidence that some older, larger fish spawned first and also that plaice in deep water spawned later than those from shallower depths. At the latter depths light was a possible factor.

According to samples from St. Mary's Bay the development of the sex products for spring spawning was apparently controlled by water temperatures during the previous fall and late summer.

Fifty per cent of the females from Flemish Cap were mature at 7.8 years, the bottom temperature being almost constant at 3.5 C. For other areas the number of years ranged from 12.2 for the Southwest Grand Bank, with prevalent temperatures 0–3, to 15.2 C for St. Mary's Bay, where temperatures were –1 to 1.0 C. For males the range was from 5.3 years for the Southeast Grand Bank to 7.5 years for St. Pierre Bank, no data being available for Flemish Cap.

INTRODUCTION

In this paper the spawning time and age at maturity of American plaice (*Hippoglossoides platessoides* (Fabr.)) are described from samples taken from waters adjacent to Newfoundland and from the Grand Bank and Flemish Cap.

Huntsman (1918) and Bigelow and Schroeder (1953) consider in a general way the breeding biology of this species principally from the Gulf of St. Lawrence, Bay of Fundy, and the Gulf of Maine. A recent paper by Powles (1965) on this species in the Magdalen Shallows deals with spawning and sexual maturity. Little has been published on the spawning and maturity of the species from the Newfoundland area. In a previous paper (Pitt, 1964) on the fecundity of American plaice from the Newfoundland area, there was a brief description of the size and age of the species at sexual maturity.

Random samples were secured from the various areas (see Table II) primarily to secure otoliths for age determination. The sex and stage of maturity were also recorded for each specimen examined. Most of the approximately

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2000 specimens considered in the preparation of this paper were checked by the author for the maturity stage. Since many of the samples were examined at sea it was not always possible to secure weights of individual fish and gonads.

The name of areas, including the various parts of the Grand Bank (Fig. 1), are used as in a previous paper by the author (Pitt, 1963).

SPAWNING

American plaice like other flatfish are bottom dwellers, but this does not mean that they do not at times rise some distance from the bottom. Bigelow and Schroeder (1953) report the catching of one in a gill net at 5–10 fath (9–18 m) where the water was 50 fath deep (91 m). Two specimens 61 and

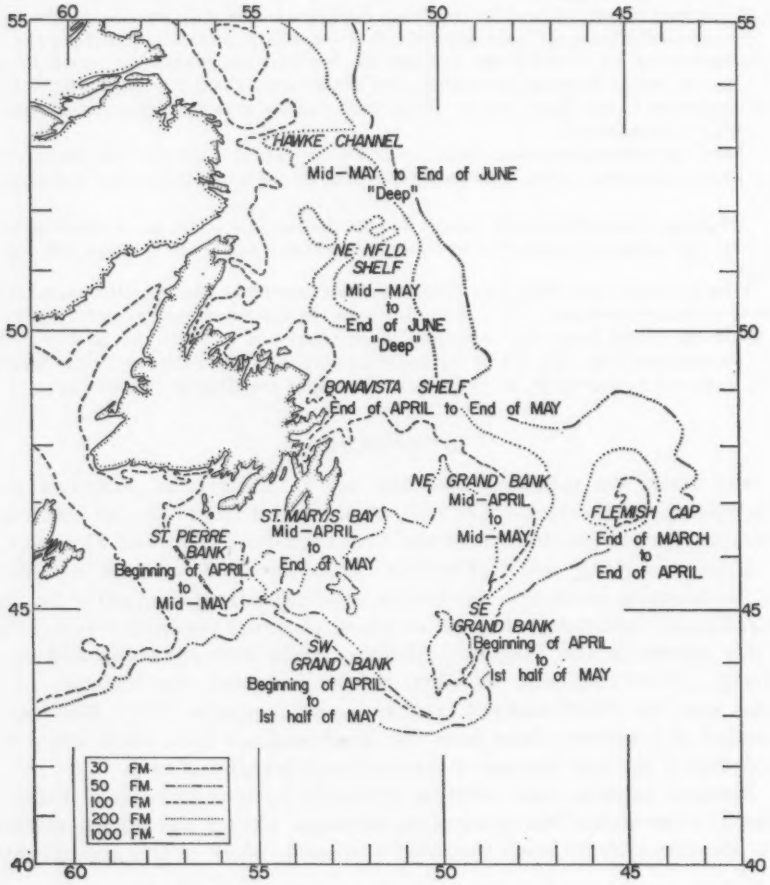

FIG. 1. Areas referred to in the text with the estimated spawning dates for these areas.

48 cm were taken on a floating trawl at 5–10 fath (9–18 m) over a bottom depth of 84–86 fath (154–157 m) by a research vessel of this Station. There was also strong evidence from day and night fishing with the otter trawl that American plaice make vertical diurnal movements (unpublished data, St. John's Biological Station). However, during daylight hours they are generally on the bottom. Huntsman (1918) stated that the plaice spawn at or near the bottom, but he apparently had no concrete evidence of this.

The Maturity Cycle

The reproductive stages as defined (Table I) are similar to those used by Powles (1956) for Magdalen Shallows plaice with one important exception. In the field description of the condition of the gonads the various maturity

Table I. Descriptions of the reproductive stages of American plaice as used in this investigation.

Male	Female
Stage I. Immature	
Testes very narrow and translucent. Vas deferens small and very thin walled.	Ovaries small, thin walled and transparent. Eggs not visible to the naked eye.
Stage II. Resting, maturing, or ripening (next year)	
Testes grey to white. No indication of previous spawning.	Eggs visible with the naked eye but no evidence of previous spawning.
These stages found after the completion of spawning, from about September to December.	
Stage III. Maturing or ripening (present year)	
Testes full and white. Vas deferens wide with comparatively thick walls, containing no milt.	A. Eggs all clearly visible with the naked eye. Ovary membrane thin. Eggs all opaque. No previous spawning. Egg diameters 0.7–0.9 mm. B. Less than one half of the bulk of the eggs clear, the rest opaque. C. Ripe. More than half of the eggs clear.
Stage IV. Partly spent and spent (present year)	
Testes white and flabby. Vas deferens wide and full of milt.	Ovary wall thickened, bluish grey on the outside, deep orange or cherry internally. Sometimes many old clear eggs present, especially in the posterior tip of the ovary. New eggs not visible to naked eye.
Stage V. Spent with recovery	
Vas deferens full of milt. Testes brownish grey, and somewhat translucent at the outer half.	New eggs becoming visible to the naked eye as the stage gradually evolves from the previous one. Previous spawning usually indicated by thick ovary wall and old eggs. This stage is sometimes indistinguishable from immature stage.

stages were related to the calendar year: for fish designated as "maturing" or "spent," the maturity condition was suffixed by "P" for present year, "N" for next year, or "L" for last year.

In determining the stage of sexual maturity there was always the problem of deciding if the gonads were maturing for the first time or had recovered from a previous spawning. Usually, this was very difficult to determine in the smaller sizes of mature females after late July and early August, unless residual eggs were present from the previous spawning. Therefore, Stage II (Table I) may have contained, besides fish maturing for the first time, some that had recovered from a previous spawning. Plaice of St. Mary's Bay were used to illustrate the half-monthly distributions of most of these stages (Fig. 2). The distributions were roughly the same for other areas except for the April–June period, when there were some variations between localities. This is considered in detail in a later section.

The presence of milt in the vas deferens was generally used as an indication that males were spent or partly spent. For some specimens examined the milt was probably forced down into the vas deferens from the ripe testis by the rough handling that the fish received in the net and during storage in ice. Thus, the percentage of males in the spent and partly spent category (Stage IV, Fig. 2) was probably exaggerated.

Residual milt was usually found in the mature males for several months after spawning, but in the females all the eggs are discharged or rather rapidly absorbed. Therefore, more males than females were put in Stage V (spent with recovery) (Table II). Thus, many males placed in Stage V may in fact have been at Stage II (maturing or ripening). The black columns in the lower panel of Fig. 2B are those males with maturing gonads that displayed no evidence of previous spawning. For the females the black columns in the lower panel of Fig. 2A include both females that were maturing for the first time and those that had recovered from the previous spawning but showed no definite proof of this spawning, i.e. the presence of residual eggs. The June and July Stage II females (Fig. 2A) were probably all maturing for the first time.

In females there was evidence that some mature fish did not produce eggs every year, since occasionally just before the start of the spawning period ovaries were encountered that were obviously in the spent condition with no recovery noted. These were usually large specimens.

There were usually some specimens that for some reason did not spawn with the majority of the population. The author has on record two mature female plaice taken in St. Mary's Bay on August 3, 1957, that were just approaching the spawning condition and a 55-cm female taken October 8, 1964, in an advanced ripe condition that would probably spawn in a week or so. These late-spawning specimens make up such a small proportion of the total population that they would have little influence on the determination of the spawning period.

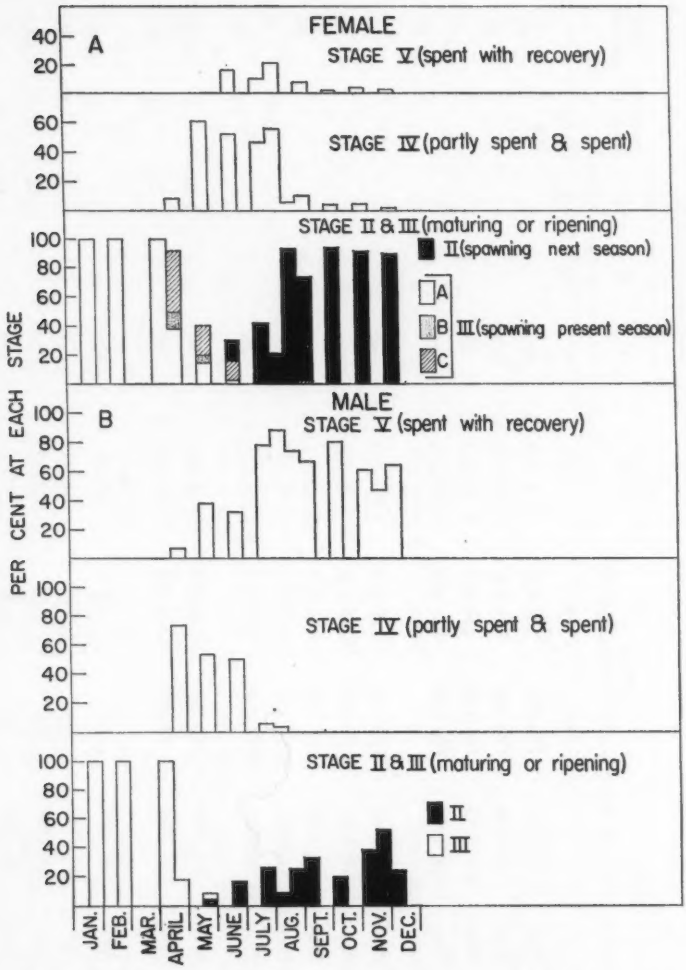

FIG. 2. Half monthly distributions of maturity stages of American plaice from St. Mary's Bay (1957–58).

SPAWNING PERIODS BY AREA

In determining the spawning time, only the females were used, because, as previously noted, some of the males that were recorded as "spent" or "partly spent" (Stage IV) may have been in the "ripe" (Stage III) condition. Since there must be males to fertilize the eggs, only the females are important in determining the spawning time.

TABLE II. Distributions of various spawning stages for samples of female plaice taken during the spawning season.

Area and latitude	Sample No.	Date	Depth		Bottom temp	Maturing, Stage III			Spent Stage IV	Total number
						A	B	C		
			fath	*m*	*C*	%	%	%	%	
Hawke Channel 52–54°N	1	Apr. 9–15, 1963	150–250	274–458	2.6 to 3.60	100	...	...	...	84
	2	Apr. 2, 1964	170–180	312–350	3.12 to 3.50	100	...	...	...	39
	3	May 2–4, 1963	106–180	194–329	2.30 to 3.00	89	11	...	...	9
	4	May 14, 1964	106–130	194–238	1.88 to 2.84	88	6	...	6	17
Northeast Newfoundland Shelf 50–52°N	5	Apr. 6–7, 1963	119–149	217–273	3.00 to 3.16	100	...	...	...	9
	6	Apr. 17, 1963	123–129	225–236	1.98	100	...	...	...	6
	7	May 5–11, 1963	107–180	196–329	0.16 to 2.93	98	2.9	...	...	54
	8	May 17, 1964	118–124	216–227	2.80	...	...	...	100	4
	9	May 25–31, 1963	113–124	207–227	0.48 to 3.80	69	7	...	24	29
Bonavista Shelf 48°30′N	10	May 12, 1963	93–105	170–192	−1.02	36	14	43	8	65
	11	June 1–3, 1963	88–140	161–256	−1.31 to 0.15	18	...	21	61	23
St. Mary's Bay 47°N	12	Apr. 9, 1957	70	128	−1.32	100	...	...	...	18
	13	Apr. 23, 1958	70	128	0.74	77	8	8	8	40
	14	Apr. 25, 1959	70	128	−1.20	13	15	63	10	67
	15	May 21, 1958	70	128	1.52	16	5	12	67	57
	16	May 29, 1957	70	128	−0.51	13	3	33	50	30
	17	June 4, 1960	70	128	−0.28	4	4	9	83	23

17	June 4, 1960	70	128	0.28						
Flemish Cap	18	Mar. 20–25, 1961	100–150	183–274	3.4	100	...	...	...	26
Northeast Grand Bank 47–48°N	19	Mar. 20–28, 1961	99–150	181–274	1.15 to 2.29	100	...	...	...	47
	20	Apr. 4–12, 1964	50–60	91–110	−0.5 to −1.0[a]	26	...	29	45	45
	21	May 18, 1953	57–58	104–106	−1.5 to 0	11	2	11	76	45
	22	May 20, 1964	98–150	179–274	1.1	26	8	32	34	38
	23	June 15, 1963	44	81	−0.33	...	...	...	100	6
Southeast Grand Bank 43–44°N	24	Apr. 23–29, 1951	26–30	48–55	0.8 to 1.6	21	5	5	9	38
	25	Apr. 24–30, 1965	80–100	...	0.8 to 1.4	19	6	21	54	84
	26	May 14, 1951	36–40	66–73	None taken	...	...	...	100	20
	27	June 12–14, 1963	35–85	63–155	−0.7 to 1.4	0	2	3	94	138
Southwest Grand Bank 42–45°N	28	Mar. 25–31, 1950	49–50	90–91	2.80	83	6	3	...	28
	29	Apr. 13–14, 1953	40–43	73–79	3.0	7	42	26	26	31
	30	Apr. 29, 1960	35–50	63–91	0.96 to 3.0	33	6	8	53	51
	31	May 7, 1963	50	91	0.7	17	11	22	50	18
St. Pierre Bank 45–47°N	32	Mar. 25–26, 1950	60	110	...	100	...	...	...	41
	33	June 2–4, 1953	65	119	...	...	...	...	100	12

[a]Temperatures taken 2 weeks later by research vessel in approximate position.

HAWKE CHANNEL. Up to the middle of April mature females were all Stage IIIA (all eggs opaque) (Table II). The next samples were for the May 2–14 period when a majority of the mature females were still at Stage IIIA. These were all comparatively deep-water samples, 106–250 fath (194–457 m), and at these depths bottom temperatures in April and May (1963 and 1964) ranged from 1.88 to 3.60 C.

NORTHEAST NEWFOUNDLAND SHELF. Spawning apparently began towards the middle of May but up to the end of May most female specimens taken still had not begun to release their eggs. Bottom temperatures for the latter part of May were 0.48–3.80 C at 113–124 fath (207–226 m).

BONAVISTA SHELF. American plaice in this area apparently spawned earlier than those to the north, since by May 12, 1963, 43.0% of the females were in the advanced ripe condition (Stage IIIC) and 7.7% had completed spawning. The bottom temperature was −1.02 C at 93–105 fath (170–192 m). By June 1–3 most of the females were spent or partly spent. At this time bottom temperatures were −1.31 to 0.15 C at 88–140 fath (161–256 m).

ST. MARY'S BAY. The bulk of the spawning occurred between the middle of April and th end of May with bottom temperatures averaging between 0 and −1.0 C at 70 fath (128 m).

FLEMISH CAP. Spawning time was doubtful but possibly occurred in April at temperatures around 3.5 at 80–100 fath (146–183 m).

NORTHEAST GRAND BANK. A sample taken on March 20–28 in this area had none of the females beyond Stage IIIA. Earliest spawning occurred during April 4–12, 1964, when most were either in the ripe state (Stage IIIC) or spent. This sample was from a commercial trawler fishing at 50–60 fath and no bottom temperature was taken; but temperatures taken 2 weeks later in this locality were all less than 0 C, so that −0.5 to −1.0 C was probably the bottom temperature. On May 20, 1964, a sample from 98–150 fath still had 26.3% of the females with ovaries containing opaque eggs.

SOUTHEAST GRAND BANK. The main spawning period here was apparently from early in April to the middle of May with bottom temperatures usually averaging around 1 C. The bulk of the spawning probably occurs at 35–50 fath (64–91 m) since this is the depth at which plaice are usually concentrated during the spring months in this area.

SOUTHWEST GRAND BANK. Spawning in this locality apparently occurred at about the same time as in the previous area, principally at depths ranging from 35 to 60 fath (64–110 m) and apparently at 0.7–3.0 C.

ST. PIERRE BANK. Spawning here probably occurs at a period similar to the two previous areas. Unfortunately, no April or May samples were obtained but from research vessel surveys temperatures at about 60 fath (110 m) are generally 0.5–3 C.

The 1963 samples for Hawke Channel, the Northeast Newfoundland Shelf, Bonavista Shelf, and the Southwest Grand Bank (Table II) gave a comparison of the time of spawning and the environmental conditions covering a large proportion of the area for a single year. During the first part of May

at Hawke Channel and on the Northeast Newfoundland Shelf, nearly all of the ovaries examined had opaque eggs, and thus the females had not spawned. Bottom temperatures were 2.30–3.00 C at 106–180 fath (194–329 m) for the former area and 0.16–2.93 C at 107–180 fath (196–329 m) for the latter. To the south of this, on the Bonavista Shelf on May 12, 1963, with bottom temperature of –1.02 C at 93–105 fath (170–192 m), most of the ovaries examined either had translucent eggs or were spent. At about the same time, May 7, on the Southwest Grand Bank 50% were spent and most of the remainder in an advanced ripe condition (Stages IIIB and C). Bottom temperature at 50 fath (91 m) was 0.7 C.

Annual Variation in Time of Spawning

The percentages of mature fish with gonads at various stages of maturity for samples taken April 23, 1958 (Sample 13), and April 25, 1959 (Sample 14), gave an indication of the annual variation in the spawning time in St. Mary's Bay. In 1958, only 7.5% of the females had ovaries in the advanced ripe state (Stage IIIC) whereas in 1959, 62.7% were at this stage (Table II). In 1958, 77.5% of the females had no clear eggs in the ovaries (Stage IIIA) but in 1959 only 14.9% were in this condition. Water temperatures were about 2 C degrees lower in April 1959 than at a comparable date in 1958 (Table III), despite the indication that the 1959 spawning occurred almost 2 weeks earlier than in 1958.

In the summer and fall of 1957, preceding the 1958 spawning, bottom temperature in the bay was probably below 0 C and in December it was only 0.06 C (Table III). In a similar period preceding the 1959 spawning, bottom

Table III. Sea temperatures (in degrees centigrade) for St. Mary's Bay for certain months, 1957–59 (only months for which temperatures were available).

		1957			1958				1959
Fathoms	Metres	June	Aug.	Dec.	Apr.	July	Aug.	Nov.	Apr.
0	0	6.25	13.39	3.32	3.13	12.87	14.69	7.06	0.29
5	10	...	...	...	...	...	...	...	–0.12
15	25	5.70	4.62	3.16	1.65	3.38	9.74	7.06	–0.29
27	50	0.74	1.15	3.10	0.93	1.24	1.70	6.99	–0.72
41	75	–0.48	2.28	3.83	0.53	0.92	1.08	2.12	–1.20
55	100	–0.27	0.06	2.12	0.58	0.88	2.46	1.02	–1.23
71[a]	130	–0.57	–0.44	0.06	0.74	0.67	0.35	1.93	–1.26

[a]Bottom depth.

temperatures were all higher (1.93 C in November 1958). Since these were the temperatures at which the gonads were maturing for the next spawning, water temperature several months prior to spawning probably had an important influence on the timing of the actual spawning period. This does not mean that the water temperature on the spawning ground at the time of spawning may not influence the spawning period. The 1959 spawning in St. Mary's Bay occurred at temperatures as low as −1.26 C, but the gonads were apparently well developed because of the higher temperatures in 1958. Thus, the ovaries ripened earlier and spawning occurred in what is considered to be an extremely low temperature.

Spawning Grounds

No specific spawning ground for this species was recognized. Because a large proportion of the area is covered by drift ice from the middle of March to late May, it was impossible to secure specimens from all parts during the spawning season. A study of logs from commercial trawlers and limited research vessel data did not reveal any unusually large concentrations of American plaice during the spawning period from any particular locality. This does not preclude the possible existence of certain areas where a combination of environmental conditions, such as type of bottom, temperature, and depth, is especially suitable for the spawning activity of this species. Most of the northeastern and eastern slopes of the Grand Bank support the largest concentrations of American plaice in the Northwest Atlantic and these regions also are areas of greatest spawning activity.

From many surveys of the Northwest Atlantic by research vessels operated by this Station it was evident that spawning of American plaice was widespread, with the Grand Bank and to a lesser extent the southern half of the Gulf of St. Lawrence supporting the main spawning populations. American plaice also spawn on Hamilton Inlet Bank, the Northeast Newfoundland Shelf, St. Pierre Bank, and the banks off Nova Scotia. In addition, it is likely that plaice spawn on the remainder of the continental shelf from Davis Strait southward along the Labrador coast and around the coast of Newfoundland and into most of the colder bays, principally at depths to about 100 fath (183 m).

Depth and Temperature Conditions

Vertical temperature profiles for a number of hydrographic stations during July and August have been compiled for a number of years by the St. John's Biological Station (Fig. 3). These temperature profiles do not give the temperature at which the fish live for the whole year. However, July–August was the only period for which complete data are available and they do give a general picture of the temperature regime of the area at a time when the more active growth period occurs (unpublished data, St. John's Station).

In the Hamilton Inlet Bank section (Fig. 3, Line I) water temperatures of less than 0 C extended from about 25 fath (42 m) to about 100 fath (183 m).

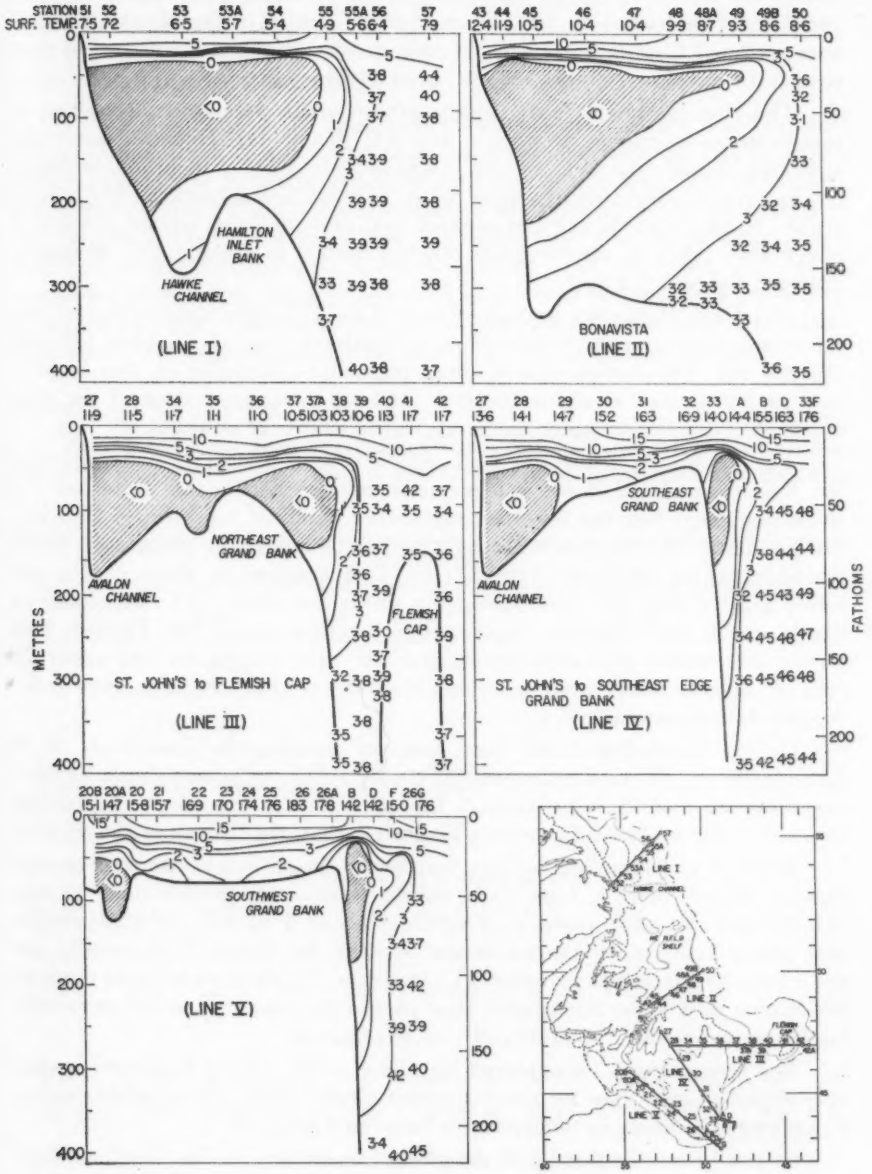
STATION 51 52 53 53A 54 55 55A 56 57
SURF. TEMP. 7·5 7·2 6·5 5·7 5·4 4·9 5·6 6·4 7·9
HAMILTON INLET BANK
HAWKE CHANNEL
(LINE I)
43 44 45 46 47 48 48A 49 49B 50
12·4 11·9 10·5 10·4 10·4 9·9 8·7 9·3 8·6 8·6
BONAVISTA
(LINE II)
27 28 34 35 36 37 37A 38 39 40 41 42
11·9 11·5 11·7 11·1 11·0 10·5 10·3 10·3 10·6 11·3 11·7 11·7
NORTHEAST GRAND BANK
AVALON CHANNEL
FLEMISH CAP
ST. JOHN'S to FLEMISH CAP
(LINE III)
27 28 29 30 31 32 33 A B D 33F
13·6 14·1 14·7 15·2 16·3 16·9 14·0 14·4 15·5 16·3 17·6
SOUTHEAST GRAND BANK
AVALON CHANNEL
ST. JOHN'S to SOUTHEAST EDGE GRAND BANK
(LINE IV)
METRES
FATHOMS
208 20A 20 21 22 23 24 25 26 26A B D F 26G
15·1 14·7 15·8 15·7 16·9 17·0 17·4 17·6 18·3 17·8 14·2 14·2 15·0 17·6
SOUTHWEST GRAND BANK
(LINE V)
LINE I
LINE II
LINE III
LINE IV
LINE V

FIG. 3. Average temperature profiles for most of the study areas from July and August 1959–63 (adapted from May et al., 1964).

Below this from 100 to 150 fath (183–274 m) on the seaward slope the temperatures were from 1 to 3 C. From a survey cruise to the Hawke Channel area in April 1963 temperatures at this depth were 2–3 C, indicating that temperatures below 100 fath (183 m) probably vary little.

The ripe specimens from these northern areas were generally taken at temperatures averaging between 2 and 3 C (Table II). However, after the spawning period, the bulk of the comparatively small population probably moves into shallower depths with lower temperature conditions. On several cruises to this area during the summer and early fall the largest catches of plaice were taken at temperatures ranging from 0 to about 1.5 C, at depths of about 100 fath (183 m).

The Cape Bonavista section (Fig. 3, Line II) gave <0 C water of the Labrador current on the coastal slope at depths of from 25 to about 125 fath (46–229 m). The comparatively small population of plaice in this locality is probably located at about 100 fath (183 m) throughout most of the year and is thus exposed to water temperatures below 0 C at all times.

The St. John's to Flemish Cap section (Fig. 3, Line III) indicated that the Northeast Grand Bank was generally covered with <0 C water on the shallowest parts but the bottom temperature observed for Flemish Cap was around 3.5 C. On the northeast part of the Grand Bank plaice were most abundant at 60–100 fath (110–183 m). Temperatures at these depths are rarely greater than 0 C and during the spring are about −1 C, especially at depths to 80 fath (146 m). Hydrographic sections across the Flemish Cap during late winter and early spring indicate that, except for the upper 20 fath (37 m), the temperature condition is similar to that for the average July–August data shown in Fig. 3.

On the Southeast Grand Bank most of the samples came from 25–50 fath (45–91 m). In this locality and at these depths the average summer temperatures are from 0 to 2 C (Fig. 3, Line IV). During the winter the volume of water below 0 C would generally be greater than that shown in the section.

Most of the sampling for the Southwest Grand Bank occurred around Station 26–26B (Fig. 3, Line V) at depths usually not greater than 50 fath (91 m) and at temperatures ranging from about 1 to 3 C. It was possible that plaice living along the southwest slope of the Grand Bank usually did not encounter temperatures below 1 C. However, if they were to move towards the central part of the Bank for at least part of the year they would encounter lower temperatures.

The temperatures encountered by plaice on St. Pierre Bank were probably slightly lower than for the Southwest Grand Bank, the spawning population generally living in temperatures between 0 and 3 C.

Generally speaking, most of the plaice populations in the Newfoundland area live at comparatively shallow depths of less than 100 fath (183 m). These are principally the north, northeast, east, and southeast parts of the Grand Bank. While it is possible that some of these fish move into deeper and thus

warmer water during the winter and early spring, most of the population probably remains at temperatures of −1 to 2.5 C throughout the year.

Spawning Time in Relation to Size

In a cod survey of the area from Hawke Channel to the northern part of the Grand Bank it was found that the larger fish were the last to spawn (Anon., 1964). For American plaice no such relationship was noted and indeed the data in Table IV show some indication that the contrary is the case, with the largest fish being the most likely to spawn first. This might merely be a function of size and previous spawning history since the larger size ranges contained increasingly larger proportions of fish that had spawned previously. Templeman (1948) also found that the largest capelin were the first to spawn and Alm (1959) reports similar conditions in certain freshwater fish.

Spawning in Relation to Depth

Specimens from the Northeast Grand Bank (Table II, No. 20) from 50 to 60 fath (91–110 m), April 4–12, 1964, were at about the same spawning

Table IV. Percentages of mature American plaice of different size groups with gonads in various maturity stages.

Male					Female					
	Stage					Stage				
Length group	III (Ripening)	IV (Spent)	V (Spent with recovery)	Total no. fish	Length group	IIIA (Ripening)	IIIB	IIIC	IV Spent	Total no. fish
cm					*cm*					
St. Mary's Bay — April 25, 1959										
Up to 25	32.4	59.5	8.1	37	up to 45	18.2	13.6	50.0	9.0	21
26–31	8.2	83.7	8.1	49	46 to 50	13.8	13.8	65.5	6.9	29
32–37	2.4	84.1	13.4	82	51 and up	11.8	17.7	70.5	...	17
Bonavista Shelf — May 12, 1963										
Up to 31	11.8	32.3	55.9	34	up to 45	62.5	4.1	29.2	4.1	24
32 and up	0.0	33.3	66.6	36	46 and up	29.5	9.5	51.2	9.8	41
Northeast Grand Bank — April 4-12										
Up to 37	13.0	87.0	...	15	up to 45	30.0	...	20.0	50.0	20
38 and up	4.0	48.0	48.0	25	46 and up	20.0	...	36.0	44.0	20

condition as those from a sample (No. 22) taken a month later, May 20, 1964, at 98–150 fath (179–274 m). The bottom temperatures were apparently −0.5 to −1.0 C at the position where the former sample was obtained, but 1.1 C at the position of the latter.

Samples from Hawke Channel and the Northeast Newfoundland Shelf that were taken from depths greater than 100 fath (183 m) spawned later than those from the more southerly areas in shallower and colder water.

A comparison of deep-water samples from the northern and southern areas indicates that Sample No. 22 (Table II) from the Northeast Grand Bank taken at 98–150 fath on May 20, 1964, was at a more advanced stage than Sample No. 4 from Hawke Channel at 106–130 fath (194–238 m) on May 14, 1964.

It was previously shown that the more northerly populations spawned later than those to the south. There was not sufficient data to compare spawning periods of shallow water samples from the northern and southern areas. Except for coastal areas, Hamilton Inlet Bank with a minimum depth of from 70 to 75 fath (128–137 m) is the only shallow area off southern Labrador and it is often covered by drift ice from April to early June.

There was thus some indication that the deep-water fish spawn later than those living in shallow water and that deep-water populations to the north spawn later than those at corresponding depths in more southerly latitudes.

AGE AND LENGTH AT SEXUAL MATURITY

The age and length when equal numbers are immature and mature are commonly defined as the 50% maturing point, or M_{50}. Bliss (1935a, b, 1952), in assessing the effects of various doses of poisons and vitamins on animals, gives a method for the calculation of the LD_{50}, which in the case of the poisons is the point at which 50% of the animals are dead. Fleming (1960) used the same method to determine the age at 50% maturity of Newfoundland cod with the only difference being that in fitting the provisional line (Bliss, 1952) a better fit was obtained by using, as did Bliss, log "dosage," which in this case was the percentage mature plotted against log age on probability paper. In all cases the observed data were consistent with the fitted line at $P = 0.05$ using the Chi-square test.

The distributions of immature and mature American plaice in relation to ages and lengths are shown in Fig. 4 and 5. Not all of the areas considered in the previous section were included here. For example, the Bonavista Shelf and Flemish Cap samples had no immature males and for Bonavista Shelf females the immature fish were all small, below 35 cm, and the smallest mature specimens were greater than 40 cm. In the determination of the age at first maturity only samples taken during the spawning season were included. Because of the obvious difference in the age and size at sexual maturity the males and females are dealt with separately.

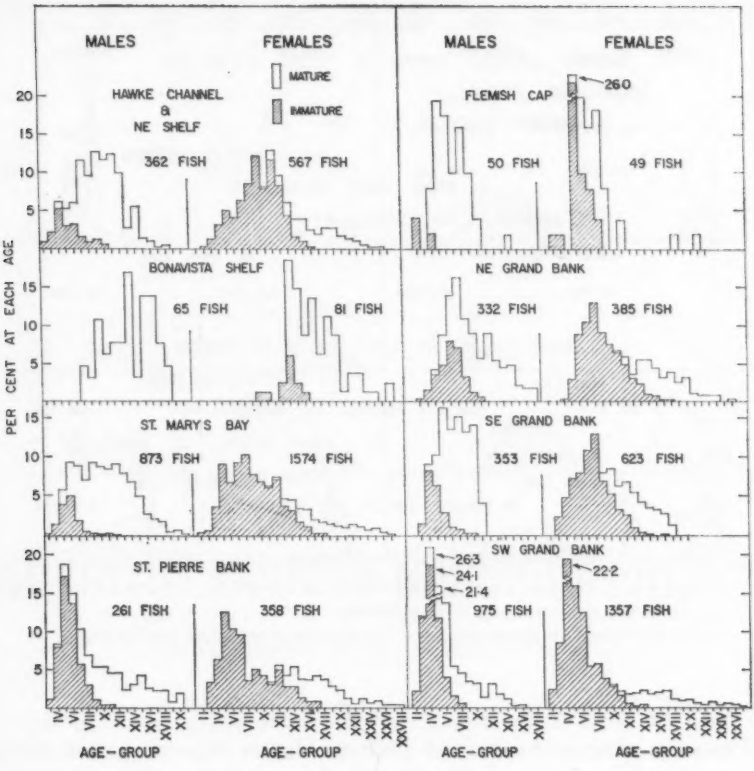

FIG. 4. Distributions of immature and mature American plaice according to age.

In areas with large fish at first maturity the sampling was probably adequate. But in a locality where sexual maturity occurred at smaller sizes the proportion of immatures may not be adequately represented since there may have been some bias in the sampling because of the very small size of some of the mature specimens (down to 14 cm for the St. Mary's Bay samples). All the catches from which the samples were obtained were taken by the otter trawl with the codend lined usually with $1\frac{1}{8}$-inch mesh braided nylon netting. For the St. Mary's Bay samples $\frac{1}{2}$-inch mesh nylon netting was used. These liners probably held all plaice that entered the codend down to the very smallest sizes. However, the net may have passed over many plaice in the lower size ranges, i.e. 15 cm and smaller, since, except in St. Mary's Bay where 6-inch rubber discs were used, the footrope was equipped with at least 14-inch rubber rollers or 18- to 24-inch steel bobbins. The latter sizes were used for all the Northeast Newfoundland Shelf–Hawke Channel sampling.

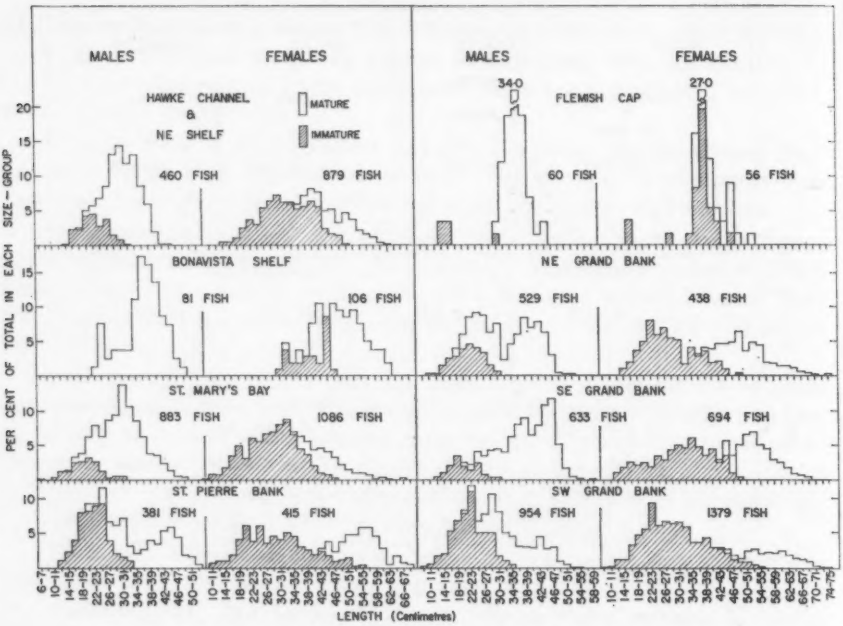

FIG. 5. Distributions of immature and mature plaice in relation to length.

AGE AT 50% MATURITY

FEMALES. Except for Flemish Cap the M_{50} for all areas ranged between 12.17 and 15.15 years (Fig. 6). The Flemish Cap population (M_{50} = 7.76 years) matured about 4 years earlier than those of the other areas ($P = 0.001$). For the Southeast Grand Bank the M_{50} (12.17) was lower than for all the remaining areas ($P = 0.001$). For the Southwest Grand Bank (13.63), the Northeast Grand Bank (13.98), and St. Pierre Bank (14.21) the values did not differ from each other ($P = 0.05$). The values for Northeast Newfoundland Shelf–Hawke Channel (14.79) and St. Mary's Bay (15.15) were not different from each other ($P > 0.05$) but were different from those for all other areas ($P = .01$). The Flemish Cap samples contained mature specimens at 6 years whereas for the remaining areas the first mature fish were found at 9–11 years. The rates at which females became sexually mature (slopes) did not differ between areas ($P = 0.05$).

MALES. The males became mature between 3 and 13 years, and the M_{50}s were heterogeneous. The range was 5.33–7.48 years (Fig. 6), but data were not available for Flemish Cap. The values for Northeast Grand Bank (7.33) and St. Pierre Bank (7.48) did not differ ($P = 0.5$) but both were higher than those for the other four areas. For these four areas only the Southeast (5.32) and Southwest (5.46) Grand Bank values were not different ($P = 0.5$).

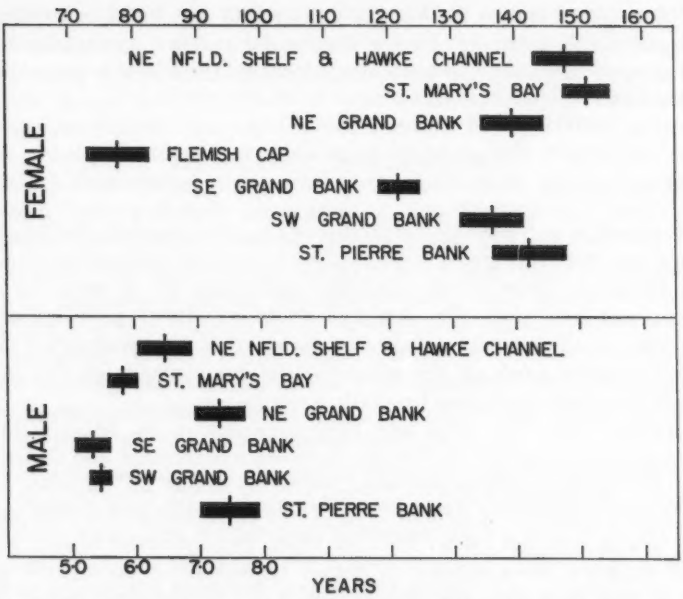

FIG. 6. Ages at which 50% of the plaice mature from various localities of the Newfoundland and Grand Bank areas. The vertical bar is the mean and the rectangle the limits of twice the standard error of the mean.

Because of sampling, as previously mentioned, there could be some error in earliest ages recorded at first maturity for some areas. The rates at which they became mature (slopes) were not significantly different between areas ($P > 0.5$ in all cases).

LENGTH AT 50% MATURITY

The lengths at 50% maturity were taken from age–length curves (unpublished data, St. John's Biological Station) using the M_{50} for age, and were listed as follows (in centimetres):

	Northeast Nfld. Shelf and Hawke Channel	*St. Mary's Bay*	*Grand Bank*			*St. Pierre Bank*	*Flemish Cap*
			Northeast	*Southeast*	*Southwest*		
Males	24.4	20.0	24.0	24.8	29.3	27.8	...
Females	42.8	40.2	42.6	46.8	53.6	44.6	41.3

The lower size ranges for the males were perhaps not adequately sampled in some areas. The length at 50% maturity for the males and females ranged

from 20.0 and 40.2 cm for St. Mary's Bay to 29.3 and 53.6 cm for the Southwest Grand Bank. Both St. Mary's Bay and Flemish Cap females began to mature at approximately the same size, although there was a great difference in their ages at comparable sizes.

DISCUSSION AND CONCLUSIONS

Time of Spawning

It is generally accepted that reproductive activity is controlled by pituitary hormones and that this gland is stimulated by certain external environmental factors. However, there is not complete agreement as to what factors are important and how they act. Atz and Pickford (1964) gave an extensive bibliography on the pituitary gland and its relation to reproduction of fishes. Most experimental work on the effects of the environment on the spawning of fish has been with fresh and brackish water forms.

Temperature. The 1958 and 1959 spawning in St. Mary's Bay gave an illustration of a possible relationship between temperature and the spawning process. The water temperatures were lower, by about 2 C degrees, in April 1959 than on a comparable date in 1958 (Table III), though the 1959 spawning occurred about 2 weeks earlier than in 1958. The apparent reason was that the 1959 spawners were subject to higher temperatures during the summer and fall of 1958 than were the 1958 spawners during the same period in 1957.

The effects of temperature on the maturing of the gonads is probably indirect, resulting from a reduction in the feeding at low temperatures. Experiments with cod by Kohler (1964) showed that food consumption and growth increase with rising temperature. He also showed that, when supplies were low, cod failed to complete the maturity process.

American plaice apparently spawned within a range of at least 4–5 C in temperature (Table II). Spawning specimens were taken from bottom temperatures ranging from −1.5 C for the Northeast Grand Bank to above 3 C for Hawke Channel, the Northeast Newfoundland Shelf, and Flemish Cap. There was no apparent indication that low temperatures necessarily retarded the spawning period; indeed, the optimum spawning temperature may possibly be 0 C or lower. This could explain, in part, the difference in the times of spawning in St. Mary's Bay in 1958 and 1959. Similarly, the Bonavista Shelf plaice taken from temperatures of less than −1 C spawned earlier than almost all of those to the north at temperatures generally in excess of 2 C. Plaice, however, from the Southwest Grand Bank spawned at temperatures of 0.7–3.0 C (Table II) at about the same period in which the Northeast Grand Bank population spawned at temperatures generally below 0 C.

Light. The effect of light on maturation of marine fishes is not as well known as that of temperature. This is perhaps partly due to the technical difficulty of measuring light at various depths. It is known, however, that fish make vertical diurnal movements, light being either directly or indirectly the causative factor. American plaice are generally bottom-dwellers, but

some apparently leave the bottom at night. This vertical movement results in fish moving into varying temperature and salinity conditions. Most of the samples examined for this investigation came from depths greater than 50 fath (91 m), so that the effects of light were obviously of less importance than in shallow coastal areas and inland streams. Blaxter (1964) gives a map indicating the probable penetration of sunlight in the Northwest Atlantic. He indicates that enough light reaches the bottom at 100 fath (183 m) to bring about light and dark adaptation of fish. Beyond this depth light at least reaches the absolute threshold value at about 200 fath (366 m).

Obviously, less light reaches the habitat of deep-water populations than those from shallower localities. Because of the greater angle of incidence of the sunlight and also because of the reduction in the length of the daylight period fron south to north during fall and winter in the northern hemisphere, the light reaching the habitats of the more northerly populations studied is possibly further reduced. During the spring the latter areas are generally covered with ice, which further reduces the light.

From a limited amount of data, it appeared that in deep water, beyond about 100 fath (183 m), mature American plaice spawned later than those from shallow water. But temperature conditions during spawning and probably during the preceding fall and winter were generally higher than those experienced by plaice that spawned in shallower water.

Both temperature and light apparently influence the spawning of plaice. It was possible that in the absence of sufficient light intensity, as in the case of deep-water populations, there was a retardation in the onset of spawning activity in spite of (otherwise) favourable thermal conditions. However, the relationship of the environment to the spawning of this species is not yet entirely understood.

Age and Size at Sexual Maturity

The species *Hippoglossoides platessoides* (Fabr.) is considered by Norman (1934) to be two sub-species: *H. platessoides limandoides* (Bloch) around the northern European coast, and *H. platessoides platessoides* on the western side of the North Atlantic. According to Bagenal (1957) the female from the Clyde population are 50% sexually mature between the second and third year at 17–20 cm. In the present study, females of the Northwest Atlantic form, on an average, were 50% mature at about 12–14 years (35–45 cm).

The Flemish Cap females (Fig. 6) matured much younger than those of the other areas. The M_{50} was 7.76 years as compared with a range from 12.12 years on the Southeast Grand Bank to 15.15 years in St. Mary's Bay. No data on the sexual maturity of males were obtained from Flemish Cap, but for the other areas the M_{50} ranged from 5.33 years on the Southeast Grand Bank to 7.48 years on St. Pierre Bank.

In a previous section of this paper reference was made to the temperature regimes of the areas under consideration and a number of temperature profiles

were illustrated (Fig. 3). By far the largest concentrations of plaice in the Northwest Atlantic are located along the north, northeast, east, and southeast slopes of the Grand Bank at depths of from 40 to 100 fath (73–183 m) and at temperatures of from about −1.0 to 2.5 C. Judging from the catches of commercial and research vessels the preferred temperatures of this species, in the areas under discussion, appear to be between 0 and 2 C. For all areas except Flemish Cap these temperatures are available without the necessity of any great migrations.

The small Flemish Cap population had little choice of temperature conditions since, as previously shown, water temperatures from about 25 fath (46 m) to about 150 fath (275 m) were constantly between 3 and 4 C. There was almost no mixing of these fish with the Grand Bank populations because of the deep channel separating the two banks. Vertebral averages from Flemish Cap were lower than those from any of the other areas considered here (Pitt, 1963), and from the earliest stages plaice in this locality were probably subjected to comparatively high temperatures.

The northern samples from Hawke Channel and the Northeast Newfoundland Shelf were generally obtained from deep water (100 fath (183 m) or greater) and at temperatures averaging about 2.5 C in April and May, but summer–autumn surveys of these areas indicate that these fish lived for part of the year in shallower and thus colder water.

Light was mentioned as a factor affecting the action of the pituitary gland on the maturation of fish. However, it did not appear to be a factor in the earlier maturation of the Flemish Cap females as compared with those from St. Mary's Bay. St. Mary's Bay, the Northeast Grand Bank, and Flemish Cap are in the same approximate latitude and the Flemish Cap samples were taken from deeper water than those of the former areas; also, light is supposed to have a stimulating effect.

It is therefore suggested that the favourable temperature conditions on Flemish Cap from even the earliest stages resulted in a fast growth rate and, as indicated by Alm (1959), a correspondingly early development and maturation of the gonads, possibly because of an earlier production of the appropriate hormones by the pituitary gland.

Another possible explanation for the much earlier maturation of the Flemish Cap population was that a genetical difference existed between it and the other populations under study, since the difference between the Flemish Cap water temperatures and those for the southern half of the Grand Bank was possibly not great enough to cause such a wide divergence in the age at sexual maturity.

Though the smallest mature males were perhaps not adequately sampled, the data indicated that sexual maturity in males began much earlier than in the females — from age 3 for the Southwest Grand Bank to age 5 for the Northeast Grand Bank and the Hawke Channel–Northeast Newfoundland Shelf areas. Males were obviously smaller than females at first maturity, and it is known that small fish generally remain in shallower and thus colder

water than larger specimens. Furthermore, in areas such as the northern half of the Grand Bank, where the food supply consists usually of sand dollars and brittle stars and there was a large population, the small fish were obviously at a great disadvantage in obtaining and utilizing food in comparison with areas such as the southwest Grand Bank where the food is usually small crustacea.

ACKNOWLEDGMENTS

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Free Drip, Flesh *p*H, and Chalkiness in Halibut[1]

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ABSTRACT

The relation between free drip, flesh *p*H, and chalkiness in halibut was investigated. Free drip was found to increase continuously with decreasing *p*H in the range *p*H 6.8–5.7. Flesh *p*H varied somewhat with location in the body, tending to be higher nearer the head. The minimum flesh *p*H was reached within 24–48 hr of death and increased very slowly thereafter.

All fish with a flesh *p*H below 6.0 were chalky, those with a *p*H above 6.2 nonchalky, while in the *p*H range between 6.0 and 6.2 intermingling of chalky, borderline chalky, and nonchalky fish occurred. The use of flesh *p*H measurement as a means of classifying fish with respect to chalkiness appears to be worth consideration.

INTRODUCTION

While it has been known for some time that the water-binding capacity of fish muscle is strongly influenced by *p*H (Tarr, 1942), and although it has been considered, apparently by analogy with meat, that *p*H has an influence on formation of free drip from fish muscle (Empey and Howard, 1954; Miyauchi, 1963), the relation between *p*H and free drip does not seem to have been investigated in fish. It has been suggested that this has been a consequence of the narrow range of *p*H values encountered in the flesh of many species of fish postmortem (Empey and Howard, 1954). However, in some species a considerable range of *p*H values occurs (see review by Amlacher, 1961). Thus, in halibut values between about 5.6 and 6.8 have been observed, and it has been found that the postmortem *p*H of the flesh has a very marked influence on the denaturation of flesh proteins during storage of the fish in ice (Tomlinson et al., 1965). At *p*H values of about 6.0 or lower the denaturation was accompanied by a change in the appearance of the flesh resulting in a condition that has been termed "chalky." In addition it has been found that free drip is greater from chalky halibut than from those of normal appearance (Patashnik, 1965), but whether there is a gradual increase in drip with a decrease in *p*H or an increase below some critical value of *p*H was not established. The present paper describes the result of an investigation of the relationship between *p*H and free drip, and also provides additional data regarding the relation between flesh *p*H and the development of chalkiness in halibut. Currently, the only means of distinguishing chalky from nonchalky fish requires visual examination of a cut surface, a procedure that is unsatisfactory in practice as it is considered undesirable to cut the fish before it reaches the point

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at which it is to be filleted. A means of classification that would obviate extensive cutting would be useful, and the possibility of utilizing flesh *p*H measurement for the purpose has been examined.

MATERIALS AND METHODS

Permission to retain halibut caught incidentally during bottom trawling (dragging) for other species was granted by the International Pacific Halibut Commission, dragging for halibut being otherwise illegal in British Columbia. Each drag was of about 15 min duration. The fish were killed by a blow on the head, eviscerated and iced. Apart from the work in which fish were examined daily for changes in flesh *p*H, the storage period was set at 11 days. The selection of this period was influenced by consideration of the postmortem age of halibut landed locally (3–20 days), and the desire to avoid undue influence on *p*H and drip by bacterial action (Miyauchi, 1963). Measurements of *p*H were made by means of a combination glass electrode thrust directly into a small incision about 1 inch deep in the flesh in the position indicated in text, tables, or figures. A Metrohm Model E280A portable *p*H meter was used at sea, a Siebold Model GLD in the laboratory. Free drip was determined by a slight modification of the U.S. standard method for frozen cod fillets (Anon., 1961) in which the time of holding at 20 C was set at 2 hr. Free drip is reported as a percentage of the initial weight of the sample. Samples for drip measurement consisted of skinned fillets cut from the portion of the body enclosed by broken lines in Fig. 1, and were as nearly as possible of uniform shape, but were not of uniform weight, the latter being influenced by the size of the fish. It was thought that this procedure would give results more likely to be in agreement with those to be expected under practical operating conditions than would a procedure involving the cutting of samples of exactly equivalent dimensions. The ratio of cut surface area to weight was thus not constant. The possible influence of this variable will be returned

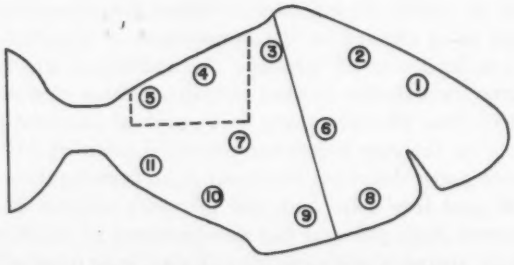

FIG. 1. Outline sketch of a halibut showing the sites at which *p*H measurements were made (numbered 1–11), the position of the cut made to enable visual examination of the flesh (solid line anterior to "3" and "9"), and the location of the position from which samples were taken for drip determinations (area enclosed by broken lines).

to below. Degree of chalkiness was judged by visual examination of the surface of a cut made across the wide portion of the body in the position indicated by the solid line just anterior to "3" in Fig. 1. Fish were rated chalky when their flesh was white, opaque, and dull in appearance; bordeline when white, nearly opaque, but shiny in appearance; not chalky when translucent and shiny in appearance. The fish examined were chiefly of small (10–20 lb) and medium (20–60 lb) weight, only a very few large (60–100 lb) fish being taken during the course of the work.

RESULTS

Variation of *p*H Within the Flesh

In preliminary experiments (Table I, Fig. 1), variation in *p*H within the flesh was investigated. It was found that there was a tendency for the *p*H to be higher near the head of the fish (positions 1, 2, and 8, Fig. 1) and lower near the center of the body (positions 6 and 7) than the average value for all positions measured (Table I). As indicated, the least variation from mean values for the whole fish occurred at positions 3, 4, 5, 9, and 10. Maximum variations in *p*H encountered between two positions were of the order of 0.3. A tendency for flesh *p*H values to be higher near the head than elsewhere in the body of fish has been observed earlier in other species (Kawabata et al.,

Table I. Variation in flesh *p*H in halibut with location of the site of measurement.

Position No.[a]	Mean *p*H[b]	Mean deviation from mean *p*H for all positions
1	6.05	+0.09
2	6.03	+0.07
3	5.96	0.00
4	5.97	+0.01
5	5.96	0.00
6	5.89	−0.07
7	5.85	−0.11
8	6.04	+0.08
9	5.96	0.00
10	5.96	0.00
11	5.87	−0.09

[a]The location of the positions are indicated in Fig. 1.
[b]Each value is the mean for seven fish.

1952). For the investigations described below, position 3 was selected as the point at which *p*H measurements were made.

Change in Flesh *p*H with Time

Although there is some scatter, it will be seen (Fig. 2) that the minimum *p*H in iced fish was reached within the first or second day of storage and that there was a tendency for the *p*H to increase slowly thereafter. A similar trend has been reported earlier for various species of fish (see reviews by Reay and Shewan, 1949; Amlacher, 1961).

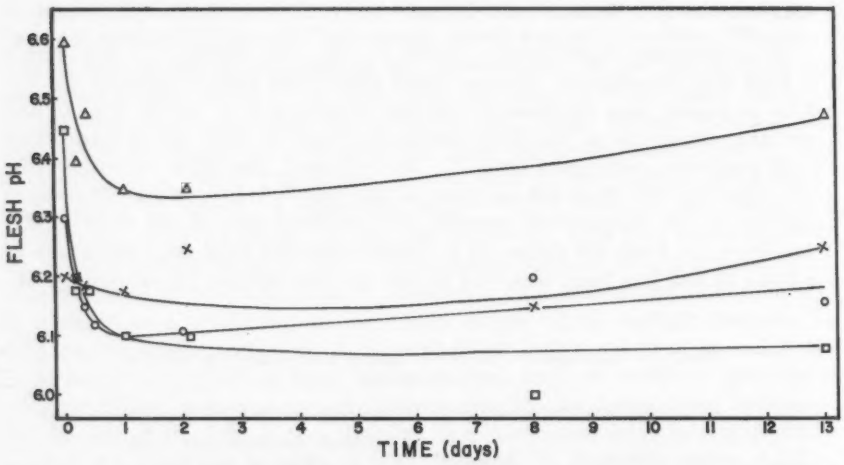

Fig. 2. Changes in halibut flesh *p*H with time of storage in ice. Each measurement was made in a separate, fresh incision in an area surrounding position "3," Fig. 1. The temperature of the flesh of these fish was 9–10 C when they were killed, and 0–1 C 4 hr after they were iced. The two small fish were in rigor within 4 hr of being killed, the medium fish within 8 hr. Small fish, △——△ and □——□; medium fish, ○——○ and X——X.

Relation Between Flesh *p*H, Free Drip, and Chalkiness

One hundred and twenty-two halibut were examined for flesh *p*H, free drip, and degree of chalkiness (Fig. 3). While there was a good deal of scatter in the free drip values observed, probably reflecting the rather numerous additional variables known to affect free drip (Miyauchi, 1963), it is clear that there was a trend toward higher values with decreasing *p*H and that this trend is continuous within the *p*H range encountered. However, on the average, after 11 days of storage in ice, free drip from chalky fish (8.5%) was a little more than double that from nonchalky (3.8%), with that from borderline fish (6.2%) occupying an intermediate position. The *p*H values of the flesh of definitely chalky fish were below *p*H 6.06 in agreement with previous observations (Tomlinson et al., 1965), while those of borderline chalky fish fell between 6.0 and 6.2 within which range, and particularly between

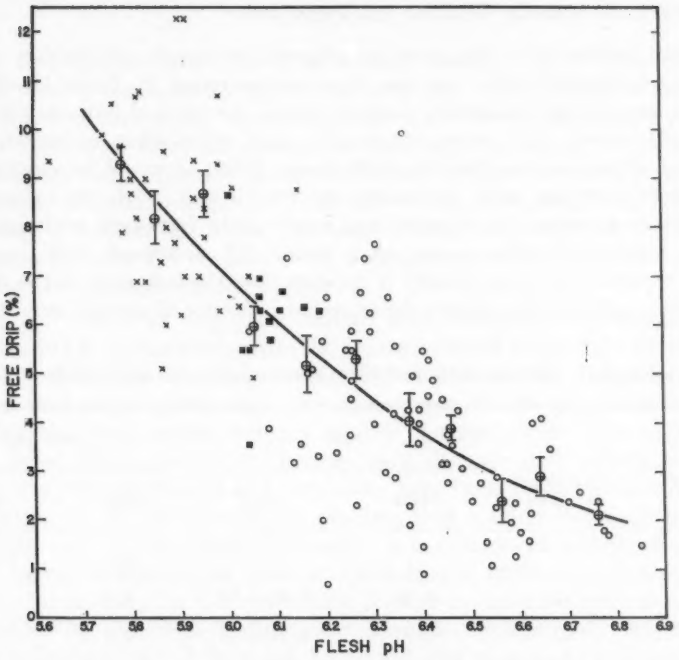

FIG. 3. Relation between free drip, flesh *p*H, and chalkiness in halibut. Free drip and flesh *p*H (position "3," Fig. 1) measurements made after the fish had been stored 11 days in ice. Chalky fish, X; borderline chalky, ■; nonchalky, ○. Mean value within each 0.1 *p*H unit interval, and the standard error of the mean, are indicated by ⊕ and the vertical line drawn through that symbol, respectively.

6.1 and 6.2, intermingling with a number of nonchalky fish occurred. This latter finding is in good agreement with that of Patashnik (1965), who found that severely chalky fish had a flesh *p*H below 6.0 while that of slightly chalky fish was between 6.0 and 6.2, in which range some nonchalky fish were also found. In the present work the flesh *p*H of only two nonchalky fish fell in the range 6.0–6.1. The values for the remainder were all higher than 6.1.

As would be anticipated from the range of flesh *p*H values found in individual fish, it has been observed during the course of this work that the flesh of chalky fish may not be uniformly chalky throughout. This has also been the experience of investigators at the Bureau of Commercial Fisheries Technological Laboratory in Seattle, Wash. (Patashnik, personal communication). In one fish examined in the present work, chalky flesh was located in a few relatively narrow bands, extending lengthwise through the body, with much wider areas of nonchalky flesh between them. The *p*H measured in the chalky flesh was 6.04, that in the normal, 6.18.

Influence of Sample Weight on Free Drip

Data pertaining to the possible influence of sample weight (i.e. cut surface area to weight ratio) on free drip are presented in Table II. Since all samples were of approximately uniform shape, the ratio of cut surface area to weight decreased with increasing weight, and, from what is known of the influence of this ratio on free drip (Miyauchi, 1963), it would be expected that drip would decrease with increasing weight. In fact, with the exception of the lightest samples, the reverse was true — drip increased with increasing sample weight. It will be seen that mean *p*H decreased with increasing sample weight, and consequently it appears that the influence of the decrease in *p*H was sufficient to obscure the expected influence of sample weight except

Table II. Sample weight and *p*H in relation to free drip and chalkiness.

Weight of samples	Number of samples	*p*H		Free drip	
		Mean	Range	Mean	Range
g				%	%
70–100	18	6.36	5.90–6.78	5.4	1.7–12.3
100–200	61	6.30	5.81–6.85	4.8	1.1–12.3
200–300	35	6.05	5.63–6.66	6.3	0.7–10.9
300–400	5	5.99	5.74–6.40	6.7	0.9–10.7

The percentage of each weight group found to be chalky or borderline chalky was as follows:

70–100 g	16.7%	200–300 g	65.7%
100–200 g	28.2%	300–400 g	80.0%

The overall percentage chalky or borderline was 41%.

with the smallest samples. It is probable, however, that differences in sample weight had the effect of reducing the rate of increase in free drip with decreasing *p*H (Fig. 3) below that which would have been observed with samples of uniform shape and weight. As mentioned previously, it seems likely, however, that the data presented approximate the trend to be expected under practical conditions. It is of interest that since the sample weights reflect the weights of the fish from which they were taken, there was a tendency toward a mean lower flesh *p*H and an increased incidence of chalkiness in the larger fish, a tendency previously observed by Patashnik (1965). Earlier, however, we have on one occasion encountered a high percentage (about 90%) of chalkiness in small fish.

DISCUSSION

The finding that free drip from halibut flesh is greatly influenced by *p*H — increasing continuously with decreasing *p*H — is in accord with expectations based on the earlier observations of Tarr (1942), and indicates that flesh *p*H may be of more importance in the exudation of free drip from fish flesh than has been generally recognized. This applies particularly to those species, such as tuna, in which very low *p*H values are encountered. However, the results for halibut also show that very substantial differences in free drip can occur within the *p*H range 6.2–6.6, a range not uncommonly encountered in various fish of commercial interest.

The fact that free drip from halibut flesh increased continuously with decreasing *p*H is in harmony with the view expressed previously (Tomlinson et al., 1965) that there is a range in degree of chalkiness that merges gradually into the nonchalky appearance. In consequence of this gradualness, while nonchalky and very chalky fish are readily distinguishable from each other by the usual visual means, it becomes very difficult to establish an exact dividing line between chalky and nonchalky fish. In addition to this difficulty, classification by visual examination requires that a fairly large cut be made in the fish, and, as mentioned previously, it is considered undesirable to cut the fish before it reaches the point at which it is to be filleted. Consequently, a means of classifying the fish that would obviate the need for extensive cutting is desirable. The use of flesh *p*H measurement for this purpose would satisfy the latter requirement if it could be established that such a measurement would provide a reasonably satisfactory means of separating chalky and nonchalky fish.

In our experience, the greatest difficulty in classifying a fish as chalky or nonchalky on the basis of visual examination of the flesh is encountered with those individuals whose flesh *p*H lies between 6.0 and 6.2, difficulty rarely being experienced with fish whose flesh *p*H is outside this range. Examination of the data presented above, together with that of Patashnik (1965) and Tomlinson et al. (1965), will show that no nonchalky fish would be classified as chalky on the basis of flesh *p*H if *p*H 6.0 were to be made the dividing point, those below *p*H 6.0 being considered chalky. However, a very small percentage of actually chalky fish and most borderline fish whose *p*H values are above 6.0 would be classified as nonchalky. On the other hand, if *p*H 6.1 were taken as the dividing point, no definitely chalky fish, and relatively few borderline fish would be likely to be classified as nonchalky, while very few actually nonchalky fish would be classified as chalky. It thus appears that, while classification by means of *p*H measurement would not be perfect, it is sufficiently promising to warrant consideration. It should be noted that the data on which the above considerations are based only cover periods of storage in ice of about 2 weeks duration, and that minimum flesh *p*H values in halibut are not reached until after about 1 or 2 days of storage.

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Pilot Plant Fractionation of Marine Oils Methyl Esters[1,2]

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ABSTRACT

A pilot-plant procedure is described whereby marine oils with low to medium iodine values can be converted into fractions of methyl esters of defined fatty acid composition. Three major fractions were obtained having 61% saturated, 73% monounsaturated, and 72% polyunsaturated esters, respectively.

Methyl esters were produced continuously and glycerol separated by centrifuge, the shorter chain-length esters distilled under vacuum and the remainder countercurrently fractionated with nitromethane. By cooling the nitromethane-rich raffinate, a highly unsaturated ester concentrate was obtained. Gas–liquid chromatography was used to follow the efficiency of the various steps.

INTRODUCTION

The bulk of fish and marine mammal oil produced in the world today is used for edible purposes after refining and hydrogenation although considerable amounts are used in paints and other coatings. Specialty uses include leather tanning and lubrication additives.

The marine oils may vary in iodine value from about 80 to more than 200 dependent on species and area of habitat. The high-iodine value oils such as pilchard and some menhaden oils can be used as drying oils with very little treatment, only winterizing being necessary to remove the stearine and sometimes followed by blowing or heat bodying. The fatty acids from these oils can be vacuum-distilled to yield fractions of very high iodine values.

Only in the last few years, since gas–liquid chromatography became available, has the fatty acid composition of marine oils been studied in detail. Fractionation procedures can now be followed closely, and this study has shown that many earlier results based on iodine values alone were very misleading.

Countercurrent distribution has been used extensively on a laboratory scale to concentrate or prepare unsaturated fatty acid esters (Scholfield, 1964), but no reports have been published on pilot-plant or industrial-scale separations. Nitromethane has been employed in combination with other solvents (Cannon et al., 1952; Lakshmanan and Laddha, 1960) and Schmid et al. (1963, 1965) have determined the critical solution temperature of several methyl esters in nitromethane. The selective solubilities of methyl esters in

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nitromethane can also be used to aid in their identification by gas–liquid chromatography (Jangaard, 1965).

EXPERIMENTAL

The methyl esters were prepared using the continuous reactor described in earlier publications (Jangaard and Vandenheuvel, 1961a, b). Sodium hydroxide at a concentration of 0.75% was employed, the methyl esters stripped of methanol under vacuum and filtered to remove the soaps. The herring oil was from Pacific herring (*Clupea pallasii*) and was obtained from the Canadian Fishing Company Ltd., Vancouver, B.C. The analytical constants were: iodine value, 135; saponification value, 190; free fatty acids, 0.9%; bound glycerol, 10.1%, and unsaponifiable material, 1.0%. The distillations were carried out in 2-liter batches at a vacuum of 1.5 mm using a 45-cm Vigreux column until 30% had been removed.

The extraction column was made of 4-inch industrial glass pipe, 6 ft high, and contained 15 stages made of Raschig Rings packed 2 inches thick between perforated metal plates. Stirring could be carried out in the zones between these packed areas by rotating the shaft (Vandenheuvel and Jangaard, 1957). The flow rates were 180 and 400 ml/min for methyl esters and nitromethane, respectively.

Samples of the methyl ester-rich top layer (A1T, A2T) and the nitromethane-rich bottom layer (A1B, A2B) were taken every 5 min. The nitromethane was evaporated in a rotary evaporator, the amount of ester and the iodine value determined, and the fatty acid composition analyzed by GLC.

The nitromethane-rich (bottom) fraction was cooled in a coldroom kept at –8 C where it separated into two layers and the fractions obtained similarly analyzed (C3B–8B, C3B–8T).

A material balance of the distillation and run "C" based on 100 lb starting material was as follows (for simplified composition of fractions see Table I): 100 lb methyl esters herring oil "A" was distilled to give 30 lb C_{12}–C_{16} fraction and 70 lb fraction "C." "C" was countercurrently extracted with 210 lb nitromethane to give a bottom layer containing 17.5 lb esters (C3B) and a top layer containing 52.5 lb esters. The nitromethane-rich bottom layer was further cooled to –8 C. On separation, the new bottom layer from this solution contained 10.4 lb esters (C3B–8B) and the new top layer 7.1 lb ME (C3B–8T).

The GLC of the methyl esters was carried out on a Barber-Colman Model 10 equipped with a model 5121 flame ionization detector, employing two glass columns (3 mm inside diameter, 6 ft in length) packed, respectively, with 11% EGSS-X and 12% EGSS-Y on 100–120-mesh Gas-Chrom P (Applied Science Laboratories, Inc., State College, Pa., USA). The operating conditions were: column temperature, 195 C, flash heater temperature, 270 C, and argon pressure, 15 psi. Tentative identifications of peaks were made by the linear log plot and separation factor procedures (Ackman et al., 1963; Ackman and Burgher, 1965) and quantification through appropriate correction factors (Ackman and Sipos, 1964).

RESULTS AND DISCUSSION

Run A was carried out without stirring until sufficient samples at equilibrium had been collected; the stirrers were then started for Run B. The agitation appeared to be very good in the areas between the packed stages where the stirrers were located. However, in spite of the increased contact, the extraction did not seem to be more efficient as judged from the iodine values, and the gas–liquid chromatographic analyses also supported the view that stirring did not increase the efficiency of the extraction in this case. In Table III the distribution of the individual fatty acids in each fraction is listed.

Starting with the shorter chain length esters, we found that 14:0 (myristic acid) was equally distributed between the two phases. The insolubility effect due to its saturation was cancelled by the effect of short chain length. With palmitic acid (16:0), a slight segregation was noticeable, although a high percentage was still soluble in nitromethane. The 16:1 was slightly more soluble and some enrichment in the bottom layer was evident. The 18:0 (stearic acid) was not soluble in nitromethane and stayed in the top layer. Oleic acid ester (18:1) was still quite soluble, although a definite enrichment took place in the top layer.

In the C_{20} and C_{22} series the difference in solubilities became quite marked. The long chain monounsaturated esters, 20:1 and 22:1, showed a sharp decrease in the soluble fraction and the amounts of the highly unsaturated 20:5 and 22:6 acid esters were approximately doubled. The increase in these two

TABLE I. Results of gas–liquid chromatographic analysis, simplified.

	Starting material A-B	A1B[a]	A2T[b]	B2B	B3T	Distilled C_{12}–C_{16}
Saturated fatty acids	27.7	20.4	29.1	18.5	28.4	60.6
Monounsaturated fatty acids	49.8	35.2	57.6	37.2	57.0	32.4
Polyunsaturated fatty acids	22.5	44.4	13.3	44.3	14.6	7.0
Iodine value	132	202	108	205	109	62
	Starting material C	C3B	C2T	C3B-8B	C3B-8T	
Saturated	8.5	4.0	8.8	3.4	8.3	
Monounsaturated	62.1	35.0	72.4	24.9	62.4	
Polyunsaturated	29.4	61.0	18.8	71.7	29.3	
Iodine value	167	261	138	300	174	

[a]B = Bottom, nitromethane-rich fraction.
[b]T = Top, ester-rich fraction.

acid esters from 10 to 20% (20:5) and from 5 to 10% (22:6) was therefore responsible for most of the difference in iodine value (109–205). Without gas–liquid chromatography we would not be aware of the fact that our "highly unsaturated" fraction with iodine value over 200 still contained 20% saturated acid ester. This fraction, re-esterified with glycerol or pentaerythritol, would not make a much better drying oil than the original oil.

Most of the saturated acid esters could be removed by fractional distillation since the principal saturated acids are myristic (14:0) and palmitic (16:0). To cool the column sufficiently to make these esters insoluble in nitromethane would be impractical since the 18:0 and some 16:0 will precipitate and impair the separation of the two layers.

TABLE II. Fatty acid composition by gas–liquid chromatography. "C_{12}–C_{18}" fraction vacuum distilled from "A–B."

Fatty acid ester	Double bond position	Weight per cent
12:0	–	0.2
14:0	–	18.0
14:1	?	0.3
15:0	–	0.9
15:1	?	T
16:0	–	40.6
16:1	9[a]	18.3
16:2	6,9	0.2
16:2	9,12	1.9
16:3	6,9,12	1.8
16:4	6,9,12,15	2.0
17:0	–	0.6
17:1	?	0.2
18:0	–	0.6
18:1	9[a]	14.0
18:2	9,12	0.3
18:3	9,12,15	0.2
18:4	6,9,12,15	0.6

[a]Other isomers may be present or predominate.

The approximately 30% of the esters removed by vacuum distillation contained 61% saturated esters (Table II). The residue, "C," was extracted with nitromethane and the soluble fraction (C3B) contained 61% polyunsaturated esters (Table IV), double the content in the starting material and 50% higher than the soluble fraction obtained from esters not distilled. By cooling fraction C3B in nitromethane to −8 C, a fraction was obtained with a polyunsaturated content of 72% and only 3% saturated esters. Since nitromethane does not freeze before −29 C, further cooling could be carried out to reduce the content of saturated and monounsaturated esters to even lower values.

For many applications, a fraction containing a high percentage of monounsaturated esters is desirable. The optimum yield of these acids in the residual

TABLE III. Summary of gas-liquid chromatography runs; fatty acid composition of fractions; "A" and "B" runs.

Fatty acid ester	Double bond position	Weight per cent				
		"A"–"B" starting material	Not stirred		Stirred	
			A1B	A2T	B2B	B3T
14:0	–	7.0	7.1	6.9	6.5	6.7
15:0	–	0.4	0.3	0.3	0.3	0.3
16:0	–	17.8	13.1	19.7	11.8	18.9
16:1	9[a]	8.9	9.5	7.9	8.4	6.8
16:2	9,12	0.7	1.5	0.5	1.6	0.6
16:3	6,9,12	0.9	1.8	0.1	1.7	0.1
16:4	6,9,12,15	0.7	2.6	0.1	3.0	–
17:0	–	0.7	0.3	0.6	0.4	0.8
17:1	?	0.1	0.1	0.1	–	0.1
18:0	–	1.8	–	2.0	–	1.8
18:1	9[a]	20.8	17.0	23.6	20.6	23.4
18:2	9,12	0.7	0.8	0.5	0.7	0.9
18:3	9,12,15	0.7	0.8	0.3	0.9	0.4
18:4	6,9,12,15	1.2	3.6	0.8	3.7	0.6
20:1	11[a]	8.8	5.1	11.7	5.1	12.5
20:2	11,14	0.1	–	–	–	0.1
20:3	5,8,11	T	–	–	–	T
20:4	5,8,11,14	0.4	0.7	0.2	0.7	0.4
20:4	8,11,14,17	0.3	0.3	T	0.3	T
20:5	5,8,11,14,17	10.5	20.6	6.4	19.8	6.9
21:5	7,10,13,16,19 ?	0.4	0.5	0.5	0.7	0.4
22:1	11[a]	10.9	4.2	13.7	4.0	13.6
22:5	4,7,10,13,16	0.1	–	–	–	0.1
22:5	7,10,13,16,19	1.0	0.7	0.6	1.1	0.6
22:6	4,7,10,13,16,19	5.0	10.5	3.6	10.7	4.0
24:1	?	0.3	–	0.6	0.1	0.9
24:5	?	–	–	–	–	–

[a]Other isomers may be present or predominate.

esters after the extraction would therefore be of interest. From Table I we can see that when the starting material was subjected to distillation a fraction with a monoene content of 73% was obtained vs. 58% when the esters were not distilled. A further enrichment could be expected if the top layer was re-extracted with fresh solvent in the column.

The calculation of liquid equilibrium and theoretical plates for a system such as we have here is practically impossible. The numerous methyl esters with different solubilities present a problem that can only at best be roughly approximated. Treybal (1963) mentions that in some cases where vegetable oils are extracted, the iodine values can be substituted for percentages as one of the coordinate scales in the liquid equilibrium phase diagram. He writes further: "The limitations of such phase diagrams must be clearly kept in mind, however. The diagrams are ordinarily determined by single-stage extractions. Since the many compounds present may have an important influence on each

TABLE IV. Summary of gas-liquid chromatography runs; fatty acid composition of fractions; "C" runs.

Fatty acid ester	Double bond position	Weight per cent				
		"C" starting material	C3B	C2T	Cooled to −8 C	
					C3B-8B	C3B-8T
14:0	–	–	–	–	0.1	0.1
15:0	–	–	–	–	–	–
16:0	–	5.4	3.8	5.9	3.0	6.1
16:1	9[a]	1.6	1.6	1.6	1.7	1.9
16:2	9,12	0.2	0.3	0.1	0.3	0.2
16:3	6,9,12	0.1	0.2	–	0.2	0.1
16:4	6,9,12,15	0.1	0.3	–	0.1	–
17:0	–	0.6	0.2	0.7	0.3	0.5
17:1	?	T	T	T	T	0.2
18:0	–	2.5	–	2.2	–	1.6
18:1	9[a]	28.5	20.6	30.2	16.5	31.5
18:2	9,12	1.2	1.5	0.9	1.2	1.1
18:3	9,12,15	0.5	1.2	0.1	1.3	0.7
18:4	6,9,12,15	0.7	4.6	0.3	5.7	0.8
20:1	11[a]	14.0	7.3	18.7	5.2	14.0
10:2	11,14	0.1	T	T	0.1	0.1
20:3	5,8,11	T	T	T	0.1	0.1
20:4	5,8,11,14	0.8	1.0	0.6	1.2	0.7
20:4	8,11,14,17	0.5	0.5	T	0.4	0.2
20:5	5,8,11,14,17	15.0	32.2	9.9	38.1	15.7
21:5	7,10,13,16,19?	0.6	1.0	0.5	1.1	0.5
22:1	11[a]	17.5	6.6	20.9	2.5	14.0
22:5	4,7,10,13,16	T	T	0.1	–	T
22:5	7,10,13,16,19	1.0	1.8	0.8	1.8	1.1
22:6	4,7,10,13,16,19	8.9	17.5	6.4	20.4	8.3
24:1	?	0.9	0.2	2.4	–	0.8
24:5	?	–	–	0.1	0.4	0.3

[a]Other isomers may be present or predominate.

other and will in any case probably distribute differently, changing the volume ratios of mixture to solvent can alter the apparent equilibria. This, of course, is not found in the case of true ternary systems. Applications of such diagrams to multi-stage extractions, or generally to circumstances different from those for which the data were gathered, may lead to considerable error."

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Depensatory Process Based on the Concept of Hunger[1]

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ABSTRACT

In a recent paper by Larkin et al. (1964) reproduction curves are considered for life history stanzas, and a depensatory process related in form to the compensatory process of Ricker (1954) is presented. In the following development the necessity of defining such functions as two parameter systems is presented. A theoretical production curve based on the concept of hunger is derived. It is also shown that under conditions of extreme predation this curve approximates Larkin's depensatory production curve. The curve is applied to data for Adams River sockeye salmon.

INTRODUCTION

Functional relationships defining reproduction curves, although generally applied to the overall adult production of a spawning population, have, for the most part, been derived under the assumption that survival through a particular stage in the life history of a fish is density-dependent and that for all other stages is independent of population density. For example, the reproduction curve suggested by Ricker (1954) assumes that the density-dependent stage is limited to fry or fingerling which are subjected to predation. Beverton and Holt (1957) developed a reproduction curve which assumes that density-dependence is confined to the larval and pre-recruit stage and results from competition for food. There is increasing evidence that for some species of salmon the fry hatch per spawner is dependent on the number of spawners. The above processes are all compensatory in the sense that the survival rate (i.e. the percentage surviving) decreases with increasing population densities.

Ward and Larkin (1964) present evidence that predation on fry and fingerling under some conditions may be completely depensatory, i.e. the survival rate increases with increasing population densities. Since the overall reproduction curve cannot possibly be completely depensatory, it becomes clear that basing a reproduction curve on theoretical considerations applied to a single stage is not generally an adequate procedure.

Larkin et al. (1964) suggest that the overall reproduction process be broken into life-history stanzas, with the output of one stanza serving as the input of the succeeding one. In their paper, reproduction curves resulting from a two-stage life history are plotted. It is of interest to note that even when one of the stages is completely depensatory, the resulting two-stage reproduction

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curve appears to be completely compensatory. If an overall reproduction curve were depensatory for low population densities, a serious problem would be posed for fisheries management agencies, since if spawning populations were reduced below a critical level the stocks would then go to extinction.

Whether or not this can be the case is difficult to determine directly, due to the inherent variability in escapement–production data on the one hand and the possibly serious consequences of studying such a critical region. The apparent approach to the problem, therefore, becomes a matter of developing theoretical models for the individual stanzas of the life history similar to those suggested by Ricker (1954) and by Beverton and Holt (1957) and of testing the validity of such models from empirical data. In the following development a theoretical model is derived under the commonly accepted principle that the feeding intensity of predators is dependent on prey density. The resulting survival rates for the prey defines a completely depensatory process.

Since the term reproduction curve is not appropriate for stanzas that are not initiated by spawning fish, we shall refer to the input–output relationship for stanzas as production curves.

RESTRICTIONS ON PARAMETERS OF A PRODUCTION CURVE

In an overall reproduction curve, if w represents the number of units of spawning adults and Z represents the number of units of adults produced, then Z may exceed w and in fact must exceed it for some values of w if a population is to be maintained. If, however, we are considering a stanza of the life history that is not initiated by spawning adults then the production Z can never exceed the initial number of individuals w. Consequently some restraints on production curves defining stanzas may be required.

In its original form the reproduction curve of Ricker (1954) is given by the equation

$$Z = we^{a(1-w)} \tag{1}$$

In this form although Z and w are in the same units, a requirement of a reproduction curve, the units are adjusted so that $Z = 1$ when $w = 1$. If we let

$$k_1 = e^a \text{ then (1) becomes} \tag{2}$$

$$Z = k_1 we^{-aw} \tag{3}$$

Here Z always exceeds w for w less than one, but if we free k_1 from the restraint $k_1 = e^a$ and substitute the restraint $k_1 \leqslant 1$ then Z will never exceed any possible value of w. In the original derivation by Ricker as applied to a single stanza (predation on fry) it is clear that the second restraint is always satisfied.

The depensatory curve of Larkin et al. is defined by

$$Z = we^{a(w-1)} \tag{4}$$

which may be written

$$Z = k_2 w e^{aw}, \text{ where } k_2 = e^{-a} \tag{5}$$

Under this definition of k_2, Z will exceed w if w exceeds one so that the upper extremities in fig. 5 and 6 of Larkin et al. have no meaning for intermediate stanzas. In fact, no positive value of k will keep Z from exceeding w for all values of w. Of course, if the stanza under consideration is not initiated by spawning adults it will be preceded by an earlier stanza. Presumably, this earlier stage may result in a maximum output say $\overline{w}$ (certainly, if it is compensatory following a Ricker curve).

If we free k_2 from the initial restraint ($k_2 = e^{-a}$) but substitute instead the restraint

$$k_2 \leqslant e^{-a\overline{w}} \tag{6}$$

then since $w \leqslant \overline{w}$ ($\overline{w}$ was the maximum possible result of the previous stage) Z will not exceed any possible value of w.

DERIVATION OF A PRODUCTION CURVE BASED ON THE CONCEPT OF HUNGER[2]

Larkin et al. (1964) state that (4) is an obvious choice for a curvilinear depensatory process since it is the converse of Ricker's compensatory process. Insofar as (4) was first suggested to define an indicated depensatory predation on salmon fry (Ward and Larkin, 1964), it would be desirable to derive a direct theoretical justification based on a model approximating the conditions observed, viz. mortalities resulting from a large population of predators tending to take a high percentage of prey at low prey densities and a lower percentage (but a larger number) at high prey densities. In view of the restraint imposed by (6) which limits the range for which the curve is defined, one might suspect that only an empirical justification could be offered. In the following development, however, some theoretical justification is indicated.

Beverton and Holt (1957, p. 118) under a modification of Ivlev's (1945) concept of hunger assume that a feeding fish of a given size has a maximum ration that it will consume in a unit of time if the food is in unlimited supply. If, then,

R = maximum ration that a single predator will take in one day,

r = actual ration that one predator takes under the existing situation of prey density,

they assume that the intensity of feeding is proportional to $R-r$, so we may let

[2]A more rigorous derivation of the basic rate equation is given in the Appendix.

$$F_1 = a(R-r) \tag{7}$$

define the daily exponential predation rate of one fish.
Let

$$N = \text{no. of prey at time } t$$

When F_1 is small[3] (and the daily exponential rate for one predator would generally be very small), the rate of exploitation, $1-e^{-F}$, may be assumed to be equal to F so that the actual ration is

$$r = F_1N = a(R-r)\ N$$

Solving for r we have

$$r = \frac{aRN}{1 + aN} \tag{8}$$

Equation (8) is equivalent to a stationary regime equation given by Rashevsky (1959) and discussed in a paper by Ivlev (1961). The equivalence to a stationary regime follows from our assumption that F_1 is sufficiently small[3] to measure directly the rate of exploitation of a single predator. We shall make no such assumption for the combined predator population. Perhaps a common limitation of both Ivlev's and Rashevsky's developments (at least, from the point of view of our requirements) arises from the fact that they do not consider the effect of the size of the predator population on the prey population. Their work, however, was concerned with a food chain involving planktonic food organisms where continuing recruitment could be assumed. We shall assume, on the other hand, that a fixed number of prey are available at the beginning of the process of predation with no further recruitment taking place during the period of predation. The relationship between the input and output of the prey population may then be derived by the following development.

Substituting (8) in (7), we have

$$F_1 = \frac{aR}{1 + aN}$$

Now if we have n predators the total predation rate on the prey will be

$$F_n = \frac{naR}{1 + aN} = \frac{b}{1 + aN}$$

where

$$b = naR \tag{9}$$

[3]In the more rigorous derivation given in the Appendix it may be noted that no restriction on the magnitude of F_1 is required.

Assuming that other mortalities on prey during the period of predation are relatively insignificant then the rate equation defining changes in prey is

$$\frac{dN}{dt} = -F_n N = -\frac{bN}{1 + aN} \tag{10}$$

Here, in conjunction with the general notation for a reproduction curve we may choose Z as our final value of N and w as the initial number of fry that are preyed on, so that w and Z will constitute the limits of integration for N. We shall let t_1 be the total number of days predation occurs (time until seaward migration, for example). Integrating (10) we have

$$\int_w^Z \frac{(1 + aN)}{N}\, dN = -\int_0^{t_1} b\, dt$$

or

$$ln(Z/w) + a(Z-w) = -bt_1 \tag{11}$$

If we let

$$k = e^{-bt_1} \tag{12}$$

then (11) may be written,

$$Ze^{aZ} = kwe^{aw} \tag{13}$$

Unfortunately, the production curve defined by (13) cannot be solved explicitly for either Z or w. A meaningful change of variable, however, produces an equation that may easily be handled. Suppose we let J represent the total prey that are consumed by the predators. Since all mortalities producing (13) where assumed to be due to predation we have

$$J = w - Z \tag{14}$$

Combining (14) with (13) to eliminate Z we have

$$w = \frac{J}{1 - ke^{aJ}} \tag{15}$$

For a given set of parameters (a, k) we may start with arbitrary values of J, compute w from (15), then $Z = w - J$ permits a plot of Z against w. If we have a set of observations on either (w,Z) or (J,w), the parameters may be computed from the following linear regression which follows directly from (14) and (15).

$$ln(Z/w) = aJ + ln\, k \tag{16}$$

Letting $Y = ln(Z/w)$, $X = J$ gives the desired regression.

In the form given by (13) and (15) the parameters a and k are both dependent on the units. Using (9) and (12), (15) may be written,

$$w = \frac{J}{1-e^{-a(nRt_1-J)}} \tag{17}$$

From our original definitions it can be seen that nRt_1 in the exponent of (17) is the total number of prey that would be consumed if every predator took a maximum ration every day. Clearly J, the total number consumed, can never exceed this value, and it can be seen that nRt_1 is actually the asymptote approached by J for large values of w. Values of J exceeding this asymptote define a meaningless branch of (17) giving negative values of w.

The above considerations suggest that the logical unit for w, Z, and J is nRt_1. Letting,

$$w_1 = \frac{w}{nRt_1},\ Z_1 = \frac{Z}{nRt_1},\ J_1 = \frac{J}{nRt_1} \text{ and } a_1 = anRt_1$$

(13) and (15) may be written,

$$Z_1e^{a_1Z_1} = w_1e^{a_1(w_1-1)} \tag{18}$$

$$w_1 = \frac{J_1}{1-e^{-a_1(1-J_1)}},\ 0 \leqslant J_1 < 1 \tag{19}$$

Even in this single parameter form no restriction is required to keep $Z < w$. Here, a_1 measures the rate at which J approaches its asymptote. In Fig. 1, Z and J are plotted against w for $a_1 = 1$ and 5. The production curve defined by Z has the asymptote,

$$Z = w - nRt_1 \text{ or } Z_1 = w_1 - 1$$

To see the relationship to Larkin's curve, (13) may be written

$$Z = we^{aw}e^{-a(nRt_1+Z)} \tag{20}$$

In case of a very heavy predation rate, nRt_1 may be very large with respect to Z over the possible range of w controlled by the preceding stanza ($w < \bar{w}$). If this is so then Z in the exponent of (20) may be omitted giving Larkin's curve (5). Considering the relative magnitude of the dominant cycle of the Adam's River sockeye run studied by Ward and Larkin (1964), one might suspect that Larkin's approximation would not apply. In the following section some evidence for this contention will be presented.

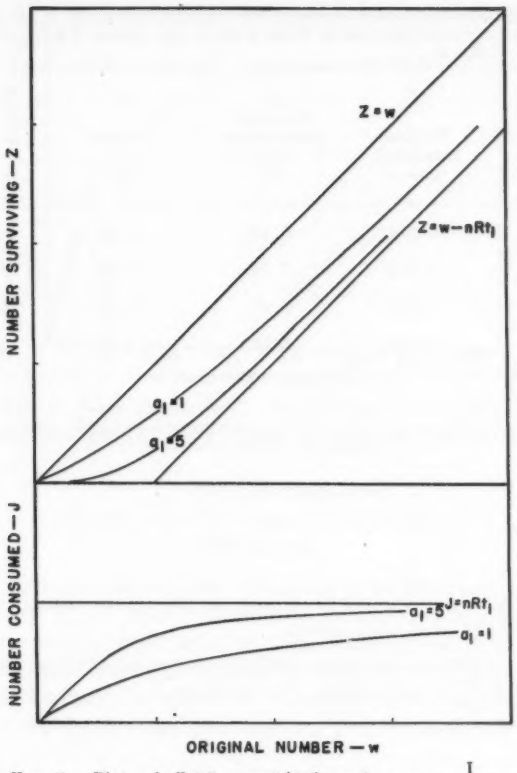

FIG. 1. Plot of $Ze^{a_1 z} = we^{a_1(w-1)}$ and $w = \frac{J}{1-e^{-a_1(1-J)}}$ for $a_1 = 1$ and 5.

APPLICATION

In table 20 and fig. 18 of the paper by Ward and Larkin (1964), data are presented relating prey density (w) and an index of the total volume of prey consumed (J). The first two columns in Table I give these data. The first column is taken from their table 20, and the second column has been read from the points plotted in their fig. 18 for the spring predation.

For the consideration of a production curve, both w and J must be in the same units. According to the preceding development, Larkin's curve is most likely to approximate the hunger curve derived here if the maximum value of Z is minimized. If we apply a factor of 7 to column (2), then in year 1 a survival rate of about 15% is indicated. This would appear to be as extreme a situation as might be permissible. Using this arbitrary factor for a scalar change on column (2) we have computed J, Z, Z/w and $ln(Z/w)$ in the succeeding columns of Table I. For the regression given by our equation (16), Y is given in column (6) and X in column (3).

TABLE I. Prey density and total volume of fish found in stomachs of all rainbow trout over 300 mm for four cycles of the Adams River sockeye run. (From Ward and Larkin, 1964.)

	(1) Initial biomass (w)	(2) Volume consumed (*index*)	(3) Adjusted consumption 7 × col. (2) (J)	(4) Survival w–J (Z)	(5) Z/w	(6) ln(Z/w)
1	0.58	0.070	0.49	0.09	.155	−1.86
2	25.48	1.025	7.18	18.30	.718	−0.33
3	65.25	1.410	9.87	55.38	.849	−0.16
4	6.10	0.680	4.76	1.34	.220	−1.51

With a correlation between X and Y of 0.937 we estimate from the regression,

$$a = 0.1992$$

$$k = 0.1225$$

Applying these parameters to equation (15) gives the curve plotted here in Fig. 2 against the observed points.

In Fig. 2 we have also plotted Larkin's curve using these same parameters in equation (5) with Z replaced by Z = w–J or

$$J = w(1-ke^{aw}) \tag{21}$$

Here, the Larkin curve can be seen to approximate (15) only for a small portion of the range of w. Of course, if we free the parameters in (21) from any restriction, a reasonably good fit can be made to the points. In this case, however, we have no theoretical interpretation of the parameters, and the fit becomes purely empirical. Further, since (21) always yields a dome-shaped curve, only the ascending limb would seem to be applicable to the type of predation considered by Ward and Larkin, so that an even stronger restriction on w than (6) would be required.

Ward and Larkin observe that the relationship between prey density and prey consumed is not linear. In their fig. 18 the plot for the summer predation as well as the spring predation both give indications of an asymptotic relationship similar to the one derived here.

THE OVERALL REPRODUCTION CURVE

A primary concern growing out of the possible existence of a depensatory stage is whether or not the resulting overall reproduction curve including such

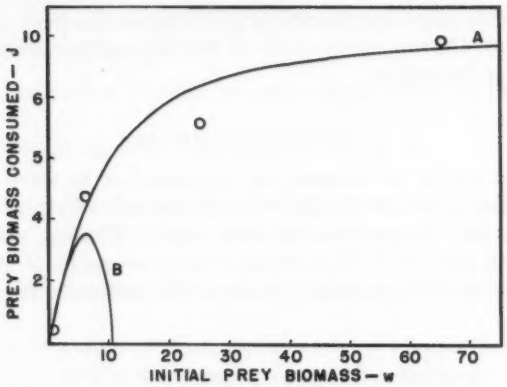

FIG. 2. Reproduction of a plot by Ward and Larkin (1964) showing relationship between prey density and total volume of fish consumed.

$$\text{Curve A is } w = \frac{J}{1-0.1225e^{0.1992J}}$$

$$\text{Curve B is } J = w(1-0.1225e^{0.1992w}).$$

a stage will be depensatory for low values of w as illustrated by Ward and Larkin's (1964) fig. 20. It is of interest to note that if the concept of hunger as developed here is combined with the basic assumption in the development of Ricker's curve (i.e. that the number of predators is proportional to the number of prey) the result is equivalent to replacing n in equation (20) by cw. It can be shown that in this case the resulting process is completely compensatory.

If we consider a compensatory process with parameters (a_1, k_1) in (3) followed by a depensatory process with parameters (a_2, k_2) then if the depensatory process follows a Larkin curve (5) it can be shown that the two-stanza curve will be completely compensatory if

$$k_1 < \frac{a_1}{a_2}$$

If the second stage depensatory process follows (13) with parameters (a_2, k_2) the resultant two-stanza curve will be completely compensatory if

$$(1-k_2)\, k_1 < \frac{a_1}{a_2}$$

Recalling that both k_1 and k_2 must be less than one it is clear that the depensatory process must be relatively severe to produce a depensatory dip for low values of w. Admittedly, since the depensatory stage considered here was the fry–fingerling period, the preceding compensatory stanza would be the

egg-stage. For this stage no theoretical justification has been given for the use of Ricker's curve. Clearly, some study of the form of the production curve for this stanza would be helpful.

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APPENDIX

Derivation of Equation (10)

Let J be the cumulative number of prey taken by n predators at time t.

Let ΔJ be the portion taken in time interval $(t, t + \Delta t)$

$\Delta J_L = nR\Delta t$ be the portion that would be taken during this period if every predator took a maximum ration

R = maximum ration that may be taken by one fish in one day.

Now during a short period of time, Δt, under Ivlev's hypothesis, fishing intensity is proportional to the difference between the maximum number of prey that could be consumed, ΔJ_L, and the actual number of prey consumed, ΔJ, or the predation rate during Δt is

$$\Delta F = a(\Delta J_L - \Delta J)$$

$$\text{but } \Delta J = (\Delta F)N$$

where N is the number of prey at time t.

Combining these last two equations

$$\Delta J = a(\Delta J_L - \Delta J)N$$

$$\text{but by definition } \Delta J_L = nR\Delta t \text{ or}$$

$$\Delta J = a(nR\Delta t - \Delta J)N$$

Dividing both sides by Δt and taking the limit as Δt approaches zero we have,

$$\frac{dJ}{dt} = a\left(nR - \frac{dJ}{dt}\right)N.$$

Assuming other sources of mortality to the prey are negligible,

$$\frac{dJ}{dt} = -\frac{dN}{dt}$$

which in the above equation gives

$$\frac{dN}{dt} = -\frac{naRN}{1 + aN} \qquad (10)$$

Partial Freezing as a Means of Preserving Various Species of Groundfish[1]

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ABSTRACT

An exploratory investigation has been made of the possibility of utilizing partial freezing as a means of preserving several species of groundfish. From the point of view of suppression of development of spoilage odours and belly burn, the procedure used was found to be superior to storage in either ice or refrigerated sea water (RSW), and the cooked texture of partially frozen fish, at least after reasonable periods of storage, was equal to that of fish stored by the other procedures. However, free drip was greater from the partially frozen fish, and fillets from such fish were softer and more fragile than those from RSW- or ice-stored fish.

INTRODUCTION

In various parts of the world a good deal of interest has been evinced in the last few years in the possibility of utilizing partial freezing as a means of improving the quality of fish delivered to the processor and the consumer, and of lengthening the storage period to which fish may be subjected without important loss of quality (e.g. Golovkin and Pershina, 1961; Scarlatti, 1964, 1965; Anon., 1964; Tomlinson et al., 1965a; Merritt, 1965). The publications indicated contain references to a few earlier investigations of the method, notably those of Le Danois (1920) and Reay (1931), which seemingly attracted no lasting interest, although the findings recorded were generally favourable.

A partial freezing procedure developed locally promises to be useful with Pacific salmon intended for canning (Tomlinson et al., 1965a). It consists of a simple modification of the refrigerated sea water (RSW) method in which sufficient salt is added to the sea water that its temperature may be reduced to between 25 and 29 F, as desired, without the storage solution becoming frozen. The salmon remain in the water for storage periods of up to 2 weeks' duration. Air must be excluded from the storage tank in order to prevent development of oxidative rancidity in the fish. The present report describes the results of several exploratory investigations of the possible applicability of this procedure, and a modification of it, to various species of groundfish of value in Canada's Pacific coast fishery.

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[2]The first author is associated with the Engineering section and the others with the Biochemistry section of this Laboratory.

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MATERIALS AND METHODS

All fish, with the exception of lingcod, were caught by dragging (bottom trawling) for 15–45 min. Lingcod were caught by hook and line and held live in sea water for 3 days before being killed. Halibut were eviscerated prior to storage, all other species were stored round. The species of fish utilized were: lemon sole (English sole), *Parophrys vetulus*; lingcod, *Ophiodon elongatus*; Pacific cod (gray cod), *Gadus macrocephalus*; Pacific halibut, *Hippoglossus stenolepis*; sablefish (black cod), *Anoplopoma fimbria*.

Permission to retain halibut caught incidentally during dragging for other species was granted by the International Halibut Commission, dragging for halibut being illegal in British Columbia.

In storage experiments conducted at the laboratory, artificial sea water, fortified with additional salt as desired, was used (Tomlinson et al., 1965a), while in experiments begun at sea, natural sea water, similarly fortified, was used. Refrigerated sea water (artificial or natural) fortified with salt is referred to as FRSW. In most of the experiments fish were also stored in refrigerated sea water or in ice for purposes of comparison. The storage apparatus used and the procedure followed in some of the experiments conducted at the laboratory have been described earlier (Tomlinson et al., 1965a). However, in a few laboratory experiments, and in all the work begun at sea, a much larger tank and chiller were utilized, and the fish were left immersed in FRSW only as long as was necessary for their temperature to be reduced to that desired, after which the water was drained off, the tank was flushed with nitrogen or carbon dioxide gas, and sealed. In one or two experiments, as indicated in the tables, the fish were cooled initially for several hours in RSW before the additional salt required in partial freezing was added to the storage solution. When the fishing vessel reached port, the sealed storage tank was transported to the laboratory and placed in a refrigerated room at the desired temperature for the remainder of the storage period. When the tank was opened for withdrawal of samples, it was again flushed with gas before being resealed. In the sampling of all species but halibut, three fish were taken from each treatment at each sampling time, and the values reported in the tables are averages for three fish. When halibut were sampled, because of the large differences encountered between individuals, particularly in flesh *p*H, six fish were taken and the values reported are averages for six fish. With the exception of halibut, all partially frozen fish were thawed in fresh water for about 2 hr at 46 F before being cut. But in view of the known *p*H-dependent reduction in solubility of muscle proteins that can occur in halibut during thawing (Tomlinson et al., 1965b), fish of this species were cut frozen, and samples for soluble protein determination were not allowed to thaw before the extraction was made.

Analytical methods have been detailed elsewhere (Tomlinson et al., 1965a,b, 1966). Weight changes in fish are reported as percentages of the initial weights; free drip as a percentage of the initial weight of the sample; soluble protein nitrogen (soluble in 5% NaCl, 0.02 M $NaHCO_3$ solution) as a percentage of the total protein nitrogen; and peroxide value as μeq peroxide

per gram of oil, the oil being that extracted from the red lateral muscle. Texture and flavour of flesh and degree of "belly-burn" (proteolysis of visceral cavity) were judged by two to four experienced members of the laboratory staff. For tasting, small samples of flesh were steamed for 10 min or fried for 6 min at 380 F.

RESULTS

A number of experiments were carried out with the various species, and the results are recorded below and in Tables I–V.

LINGCOD

Two laboratory experiments (one of which is detailed in Table I) were carried out with lingcod with similar results. Insofar as texture and flavour of the cooked flesh were concerned, there was little to choose between fish stored 19 days partially frozen in FRSW and those stored 14 days in RSW. However, in our experience, and in view of the fact that these fish had been starved for 3 days before being killed, the strong off-odours that developed in the RSW tank by the 11th day of storage, and on the fish themselves by the 14th day, indicate that under practical conditions the useful storage period for lingcod would be less than 14 days in RSW. In contrast, the slight off-odours that developed in FRSW and on the partially frozen fish in FRSW in 19 days, indicate a useful storage period in excess of 14 days for the procedure. Bacteriological examination of the flesh from RSW-stored and partially frozen lingcod on the 14th and 19th days of storage, respectively, revealed no significant difference between them (counts being less than 3×10^4 g of flesh in each case), but the count in the RSW itself had risen from 4.3×10^5 to 3.1×10^6/ml between the 5th and 14th days of storage. For description of counting procedure see Boyd and Southcott (1964). Partial freezing, however, resulted in an increase in free drip, and fillets from partially frozen fish were softer to handle than those from fish stored in RSW. Lingcod exhibited very good characteristics as regards weight changes, penetration of sodium, and loss of potassium during storage in either RSW or FRSW, particularly in contrast with Pacific cod in which these effects were much greater (see below). The course and magnitude of the weight changes during storage in RSW and FRSW were similar to those recorded earlier for salmon, but changes in flesh concentration of sodium and potassium were smaller than those observed in salmon (Tomlinson et al., 1965a). Rancidity was not detected in any of the fish examined during this particular experiment.

Transfer of the fish from FRSW to a CO_2 atmosphere when they had become partially frozen, rather than holding them in FRSW for the entire storage period, improved their flavour slightly, particularly by reducing saltiness in the red lateral muscle, but also appeared to result in a slightly more rapid decrease in protein solubility. In a second experiment fish were transferred to air rather than CO_2 and a similar impairment was not observed, but some

TABLE I. Changes in lingcod stored in RSW, partially frozen and stored in FRSW, or partially frozen in FRSW then stored in CO_2 gas.

Storage[a]		Weight change	Flesh: Free drip	Flesh: Soluble protein N	Flesh: Concentration of Na	Flesh: Concentration of K	Peroxide value	Odour, flavour, and texture
Days		%	%	%	*mg/g*	*mg/g*	*μeq/g*	
	0	–	0.8	91	0.27	4.41	1.4	
RSW	4	−0.5	1.6	93.5	0.62	3.88	–	Odour of exterior of whole fish good; of visceral cavity, slightly "off." Taste and texture (fried or steamed) good, comparable to fresh fish.
	7	−1.0	1.2	83.0	0.84	4.13	10	As at 4 days.
	14	+3.6	1.1	89.0	1.44	2.75	3.9	Odour of exterior of whole fish poor; of visceral cavity, "off" but not bad; of raw flesh, good. Flavour and texture good, but slightly fibrous steamed.[b]
FRSW	4	+0.6	5.3	95	0.56	3.92	2.2	Odour of exterior of whole fish good; of visceral cavity, like that of a freshly killed fish. Taste and texture comparable to a freshly killed fish.
	8	+1.4	4.4	89	0.85	3.77	1.3	As at 4 days.
	19	+5.7	4.4	85	1.32	3.17	0.7	Odour of exterior of whole fish and of visceral cavity slightly "off." Taste and texture good, but texture possibly a little more fibrous than that of 14-day RSW fish when steamed. Red lateral muscle salty.
FRSW CO_2	4, 8	+1.5	6.4	81	0.62	3.62	1.3	As at 4 and 8 days in FRSW at 27 F.
FRSW CO_2	4, 15	+0.2	5.2	79	0.77	3.70	2.5	Similar to 19-day fish in FRSW at 27 F, but flavour possibly a little better, and red lateral muscle not salty.

[a]Temperatures: RSW, 30 F; FRSW, 27 F; CO_2, 25 F.
[b]A bad off-odour developed in the water of the RSW tank by the 11th day of storage. Belly-burn was not observed in any of the fish in this experiment, probably because they had been held live without feeding for 3 days before being killed.

rancidity developed in the oil of the red lateral muscle. The latter development was not unexpected in view of earlier findings regarding the remarkable sensitivity of partially frozen salmon to rancidity during storage in FRSW under aerobic conditions (Tomlinson et al., 1965a). In most subsequent experiments in the present work, fish were stored in nitrogen gas after removal from FRSW.

Pacific Cod

Penetration of sodium into Pacific cod was found to be a limiting factor in storage in either RSW or FRSW, with belly-burn in round fish being a limiting factor during storage in ice, and also an important consideration in RSW (Table II). For round cod the acceptable storage period in RSW and FRSW would be less than 7 days, and probably about the same in ice, but if the fish are partially frozen in FRSW in about 2 days and then transferred to storage in N_2 gas, uptake of salt is greatly reduced and a storage period approaching 2 weeks would be acceptable, with texture changes (protein denaturation) and development of free drip becoming the limiting factors. A slight indication of an unfavourable influence of CO_2 storage on free drip and protein denaturation, similar to that with lingcod, was detected. Fillets from partially frozen fish were softer to the touch than those from nonfrozen fish.

During the course of experiment 1 (Table II), fish stored 13 days (10 days from the start of the experiment plus 3 days in ice prior to that) were subjected to accelerated spoilage by holding them for 18 hr at 8–10 C. At the end of this period the odour of the fish from FRSW (partially frozen) was better than that of fish from FRSW followed by CO_2, which in turn was superior to that of iced fish. The odour of the latter was decidedly "off." However, fillets from the partially frozen fish were considered to be "mushy" in comparison with those from iced fish and to be of poorer colour, tending to have a greenish-brown hue. The colour was more noticeable in the fish from FRSW and CO_2 storage than in those held in FRSW only, and possibly indicates a need for bleeding this species prior to storage.

Insofar as Pacific cod are concerned, it appears that evisceration at sea followed by icing or deep freezing would probably be more satisfactory than any of the above procedures utilizing round fish. Partial freezing (superchilling) of eviscerated cod at temperatures near 30 F might also be satisfactory (Anon., 1964).

Lemon Sole

Results (Table III) with lemon sole partially frozen in FRSW then transferred to N_2 gas were good in comparison with icing, although the storage period was limited to 13 days and the use of a lower storage temperature would probably have been advantageous in further reducing belly-burn. With this species, free drip was only moderately higher in the partially frozen than in the iced fish. Fillets prepared from fish after 19 days partially frozen storage,

TABLE II. Changes in Pacific cod stored in ice, in RSW, or partially frozen in FRSW and then stored in N_2 gas.

Storage		Weight change	Flesh: Free drip	Flesh: Soluble protein N	Flesh: Concentration of Na	Flesh: Concentration of K	Peroxide value	Belly-burn	Texture and flavour
Days		%	%	%	*mg/g*	*mg/g*	*μeq/g*		
Experiment 1									
Iced (controls)	3	0	3.1	91	0.46	4.68	–	None	Good
FRSW	3	+5.3	5.7	94	1.59	2.12	–	Moderate in 1, none in 2.	Texture slightly fibrous, flavour good.
FRSW	10	+8.5	6.8	79	4.92	1.40	–	Slight in 1, severe in 2.	Texture a little fibrous, not bad; flavour good, but salty.
FRSW CO_2	3 7	+3.4	10.9	77	2.00	2.17	–	Moderate in 3.	As for fish in FRSW at 10 days, but not as salty.
Iced	3	+3.5	4.8	86	0.55	1.88	–	Moderate in 2, none in 1.	Like partially frozen fish at third day.
Iced	10	+0.5	4.5	84	0.53	2.20	–	Severe in 3. Much stronger odour in visceral cavity than in partially frozen fish.	Possibly slightly inferior to partially frozen fish at 10th day.
Experiment 2									
Iced (control)	1	–	1.9	95	0.59	3.47	0	None	Good (steamed).
FRSW	1	+2.5	6.6	91	0.60	3.40	0	Slight in 1, none in 2.	Texture and flavour little if any different from fish quick-frozen first day and stored at –25 C thereafter.
FRSW N_2	1.2 0.8	+3.6	5.4	90	1.11	3.32	0	Slight in 2, none in 1.	"

FRSW 1.2 N_2 6.8	+3.4	8.0	93	1.13	3.47	0	Slight in 3.	"
FRSW 1.2 N_2 13.8	+3.3	10.5	77	–	–	0	Slight in 3.	Texture a little tougher than quick-frozen control (see above); flavour good, no indication of bacterial spoilage.
FRSW 1.2 N_2 19.8	+3.1	9.7	48	–	–	1.7	Slight in 2, severe in 1.	Texture tough, flavour good.
RSW 1	+3.4	2.7	91	1.04	3.50	–	Slight to moderate in 2, none in 1.	Same as for partially frozen fish at 1 day.
RSW 2	+5.4	1.5	89	1.40	3.00	–	Slight in 1, severe in 2.	Same as for partially frozen fish at 2 days.
RSW 7	+10.9	1.2	97	4.67	1.93.	–	Slight in 1, severe in 2.	Same as for partially frozen fish at 8 days, but salty.
Experiment 3								
RSW 0.5 FRSW 1.5 N_2 9	–	7.3	62	1.45	3.36	2.5	None in 2, slight in 1.	Texture chewy, but not bad; flavour good.
RSW 0.5 FRSW 1.5 N_2 16	–	13.3	56	–	–	6.0	None in 1, slight in 2.	Texture, fried, chewy; flavour good; steamed, texture tough to very tough.
RSW 0.5 FRSW 1.5 N_2 23	–	13.9	27	–	–	0.3	None in 1, very slight in 1, moderate in 1.	Texture, fried, better than steamed, but unpleasantly tough. Raw odour strong, but not spoiled, and cooked flavour quite good. Fillets appeared fibrous and "rough."

Temperatures:

Exp. 1: FRSW, 27 F; CO_2, 25 F.
Exp. 2: RSW, 30 F; FRSW, 27–25 F; N_2, 25 F.
Exp. 3: RSW, 30 F; FRSW, 26–25 F; N_2, 25 F.
Experiments 1 and 2 were conducted in the laboratory, experiment 3 was begun at sea.

TABLE III. Changes in lemon sole stored in ice, or partially frozen in FRSW and then stored in N_2 gas.

Storage[a]		Flesh						
				Concentration of				
		Drip	Soluble protein N	Na	K	Peroxide value	Belly-burn	Flavour and texture
Days		%	%	*mg/g*	*mg/g*	*μeq/g*		
FRSW	2	2.2	80	1.36	3.11	0	None	Flavour and texture very good (steamed).
FRSW N_2	2 5	5.9	81	1.36	2.59	0	None in 2, slight in 1.	"
FRSW N_2	2 11	4.4	76	2.59	2.39	8.5	None in 1, moderate in 2.	"
Ice	2	1.9	75	0.55	2.82	1.5	None	"
Ice	7	3.5	79	0.57	2.64	0	Some in 3.	"
Ice	13	3.2	78	0.54	2.02	32	Severe in 3.	Slight spoiled flavour, texture not as good as 13-day partially frozen fish.

[a]Temperatures: FRSW, 28.4 F; N_2, 27.5 F. Experiment begun at sea.

then frozen and held at −13 F, have proven to be very satisfactory. It is interesting that Dassow and Stansby (1952) some time ago found that lemon sole (English sole) and other "soles" were very amenable to frozen storage, and also to thawing and refreezing.

Sablefish

A few sablefish (black cod) were taken during one fishing trip and this permitted a very limited experiment to be carried out. The results (Table IV) indicate this species may be partially frozen with quite satisfactory results, although free drip was considerably increased and fillets were somewhat soft. However, it appears that sablefish must be bled before being frozen if serious discolouration of the flesh is to be avoided, and further investigation of the suitability of the partially frozen fish for smoking is needed, since this fish is usually smoked.

Pacific Halibut

It was possible to carry out only a single experiment into the partial freezing of halibut and the results (Table V) presented, which seem to show an increase in protein solubility with length of partially frozen storage, require some explanation. As will be evident from the table, it required 4 days' storage in RSW and FRSW to reduce the temperature of all the fish to 25 F, but owing to the shape of the storage tank, and the direction of circulation of the storage solution (upward through the tank), the fish at the bottom reached the desired temperature before those at the top. Consequently, from what is known of the postmortem decrease in solubility of protein in chalky fish it would be expected that the decrease would be greater in those fish nearer the top of the tank in which freezing would have been delayed longest; and it appears that this is in fact what occurred, for the fish were unfortunately and practically unavoidably sampled from the top of the tank downwards. ("Chalkiness" in halibut is a condition associated with the postmortem *p*H of the muscle. When the *p*H falls to about 6.0 or lower a very marked decrease in solubility of the muscle proteins occurs, even during storage of the fish in ice (Tomlinson et al., 1965b).) In any event, examination of the data will show that even potentially chalky halibut can be held partially frozen for 32 days without any large reduction in protein solubility occurring (in freshly killed halibut protein solubility is usually about 90% (Tomlinson et al., 1965b)) and can then be transferred to storage at −13 F for a further 2½ months without any very marked further loss in protein solubility. It is of interest that Zaitsev (1965) has recorded that only 28% of the protein of halibut was denatured during 70 days of storage at −2 C, but neither the *p*H of the flesh nor the exact method of storage of the fish was reported. It should be pointed out that the incidence of chalkiness in the fish used in this experiment was, unfortunately, exceptionally high, even for fish caught by dragging (Tomlinson et al., 1965b) and it may be possible that partial freezing tends to increase the incidence of chalkiness, for Finn (1932)

TABLE IV. Changes in sablefish stored in ice, or partially frozen in FRSW then stored in N_2 gas.

Storage[a]		Flesh					Belly-burn	Texture and flavour
		Free drip	Soluble protein N	Concentration of Na	Concentration of K	Peroxide value		
Days		%	%	*mg/g*	*mg/g*	*μeq/g*		
Iced	7	2.4	85	0.54	2.82	4	Very slight in 3.	Good.
	14	3.7	90	–	–	30	Slight in 1, severe in 2.	Texture good; flavour good in 2, somewhat "fishy" in 1.
FRSW N_2	3	7.6	96	0.41	3.49	2	None in 1, very slight in 2.	One fish very slightly chewy, others very tender. Flavour good.
	11							

[a]Temperatures: FRSW, 27–26 F; N_2, 25 F. Experiment begun at sea.

TABLE V. Changes in Pacific halibut partially frozen in FRSW, stored in N_2 gas for varying periods at 25 F then glazed and stored in air at −13 F. All values are averages for six fish, and all fish were judged to be chalky or borderline when thawed. Sodium and potassium content of the flesh of fish from 10-day storage was 0.78 and 3.48 mg/g flesh, respectively. Experiment begun at sea. Temperatures: RSW, 30 F; FRSW, 25 F.

Storage at 25 F	Free drip	Soluble protein N	Flesh *p*H	Storage at −13 F	Free drip	Soluble protein N	Flesh *p*H
Days	%	%		*Days*	%	%	
RSW 1 FRSW 3 N_2 7	14.0	55.0	5.87	73	11.3	62.7	5.97
RSW 1 FRSW 3 N_2 14	17.5	67.3	5.85	73	14.2	50.1	5.88
RSW 1 FRSW 3 N_2 21	16.3	60.0	5.81	73	15.1	56.0	5.85
RSW 1 FRSW 3 N_2 28	11.3	81.0	5.84	73	9.1	78.0	6.05

has shown that *p*H of muscle juice may be lowered slightly during freezing, but this is uncertain.

Free drip from these fish was excessive, but, curiously, tended to decrease during storage at −13 F, possibly, in part at least, as a result of a slight increase in *p*H during storage (Tomlinson et al., 1965).

Examination of cooked halibut flesh, either steamed or breaded and fried, showed quite clearly that the texture was chiefly dependent on the *p*H of the raw flesh, the lower the *p*H the tougher and drier the texture. Regardless of the storage period within the limits investigated, flesh from individual fish whose muscle *p*H was near 5.6 was very tough and dry when cooked, whereas that from fish with a flesh *p*H of 5.8–6.0, although somewhat chewy, was still usually acceptable to most tasters, particularly if it was fried rather than steamed. This has also been observed in separate work with halibut either iced or deep frozen (in dry ice) at sea, and Patashnik (1966) has reported similar observations with iced halibut. However, in the present work there was some indication that the texture of partially frozen or deep frozen fish was slightly better and the flavour somewhat better than that of iced fish of similar *p*H stored for 11 days.

Further investigation with halibut is clearly needed, both with chalky and nonchalky fish.

DISCUSSION

The results of these exploratory experiments are such as to indicate that partial freezing of groundfish is sufficiently promising to warrant further exam-

ination. In comparison with storage in ice or in RSW, partial freezing provided a very marked reduction in development of off-odours and in both the severity and incidence of belly-burn, and, during equal or somewhat longer periods of storage, yielded at least comparable cooked texture. However, it caused a marked increase in free drip, and fillets from partially frozen fish were softer and more fragile than those from iced or RSW-stored fish. The results of Reay's (1933) work with haddock and of Love's (1962) with cod, although conducted differently from the present work (their fish were initially frozen at −20 and −29 C, respectively, before being stored at the higher temperatures), suggest that these defects of the method might be overcome through the use of slightly lower temperatures. This possibility should be looked into, although the use of temperatures much below 25 F would probably be impractical with the procedure proposed. If eviscerated fish are to be stored, and prevention of belly-burn is consequently not an important consideration, temperatures between 28 and 30 F can be used to advantage (Anon., 1964). It should be noted that the defects (greater free drip and softer fillets) are much more pronounced with some species (e.g. Pacific cod and halibut, the latter at least if potentially chalky) than with others, and may be acceptable in many instances.

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A Record of *Bathypterois dubius* Vaillant from the Western North Atlantic, and Review of Status of the Species[1]

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ABSTRACT

A specimen of *Bathypterois dubius* Vaillant, 204 mm standard length, trawled by the *A. T. Cameron* at a depth of 750–805 m on the southwestern slope of the Grand Bank on May 2, 1959, is apparently the first record for the western North Atlantic. The specimen is illustrated and its characteristics described and compared with those of other *B. dubius* from the eastern Atlantic and the Mediterranean.

INTRODUCTION

A specimen of *Bathypterois dubius* Vaillant 1888, family Bathypteroidae (Fig. 1) was captured by the *A.T. Cameron* in a bottom otter trawl, May 2, 1959, on the southwestern slope of the Grand Bank, 44°18′N, 53°22′30″W, 750–805 m, bottom temperature, 3.99 C. Its colour after 5 years preservation in formalin was black. This species has been reported from the eastern Atlantic and from the Mediterranean (Vaillant, 1888; Goode and Bean, 1896; Collett, 1896; Koehler, 1896; Zugmayer, 1911; Roule, 1919; Koefoed, 1927; Fowler, 1936; Pardi, 1950; Bougis and Ruivo, 1957; Dieuzeide and Roland, 1957; Raimbault, 1963). It is also listed by Koefoed (1927, p. 14) as occurring off the east coast of North America.

I have not, however, found a reference to an actual record from the western Atlantic and the species is not included for the western Atlantic in Brauer (1906), in the check list of Jordan et al. (1930), in Grey's (1956) distribution study of *B. dubius*, nor in the more recent review by Mead (1966) of the family Bathypteroidae in the western North Atlantic. As far as we can determine, therefore, the Grand Bank specimen is the first record from the western Atlantic.

Bathypterois dubius is very variable in some of its body proportions, especially in the length of the elongated rays of the pelvic and caudal fins. There are also closely related species or subspecies and its original description by Vaillant (1888) covers only part of its range in form (Mead, MS, 1964). It was consequently necessary to study our specimen in detail both for specific identification and to provide details of body proportions and meristic characters, etc. for other workers.

The specimen, which had been preserved in 10% formalin, was measured in September 1964 after being in water for 1 hr to 1 day. It was a mature female

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with opaque eggs 0.8 mm diameter in the ovaries. The ovaries also contained several dozen clearing eggs about 1.2 mm diameter and mostly squashed. These could have been residual eggs after spawning, but in view of the large size of the opaque eggs were probably clearing eggs soon to be extruded during spawning.

The stomach contained one small mysid, *Boreomysis arctica* Krøyer, 0.1 cc.

METHODS OF MEASUREMENT

The methods of measurement of *B. dubius* by most earlier authors are not expressly stated. The standard length (S.L.) of Bougis and Ruivo (1957) and of Raimbault (1963) was measured from the anterior projection of the mandible to the base of the caudal. Although not stated it is most probable from the descriptions of Vaillant (1888), Collett (1896), Zugmayer (1911), and Koefoed (1927) that S.L. was also measured from the anterior tip of the mandible to the base of the caudal. For these authors, also, measurements involving the head were usually from the mandibular tip rather than from the snout. Unless otherwise noted my methods of measurement were those of Hubbs and Lagler (1958). I have, however, also provided measurements based on the standard length from the anterior tip of the mandible so as to provide a ready comparison with those of other authors. Usually, differences due to the different kinds of standard lengths used are not great and offer no difficulty in the comparisons made.

COMPARISONS WITH RELATED NORTH ATLANTIC SPECIES OF *BATHYPTEROIS*

Parr (1928) lists *Bathypterois pectoralis* Garman 1899 from the Pacific as synonymous with *B. dubius* but Mead (1966) points out the differences between these two species.

In considering Mead's (1959, 1966) keys to the North Atlantic Bathypteroidae, it is not possible to determine whether the scales behind the base of the pectoral fin in the Grand Bank specimen are pectinate or nonpectinate because these scales have been lost. As the specimen has a subcaudal notch, its closest North Atlantic relatives are *B. dubius* Vaillant, *B. phenax* Parr, and *B. quadrifilis* Günther.

Some differences between the Grand Bank specimen and *B. quadrifilis* are: the lowermost ray of the lower part of the pectoral is not (as in *B. quadrifilis*) conspicuously thicker than its neighbour; the point of separation of the upper two pectoral rays is well posterior to the posterior end of the dorsal (anterior to pelvic fin origin in *B. quadrifilis*); the eye is smaller; there are eight rays in the pelvic (nine in *B. quadrifilis*); the anal is well behind (ca. 8 mm) the vertical from the base of the last dorsal ray (under or slightly behind in *B. quadrifilis*).

Some differences between the Grand Bank specimen and *B. phenax* are: the nine pelvic rays in *B. phenax*; in *B. phenax* the posterior end of the

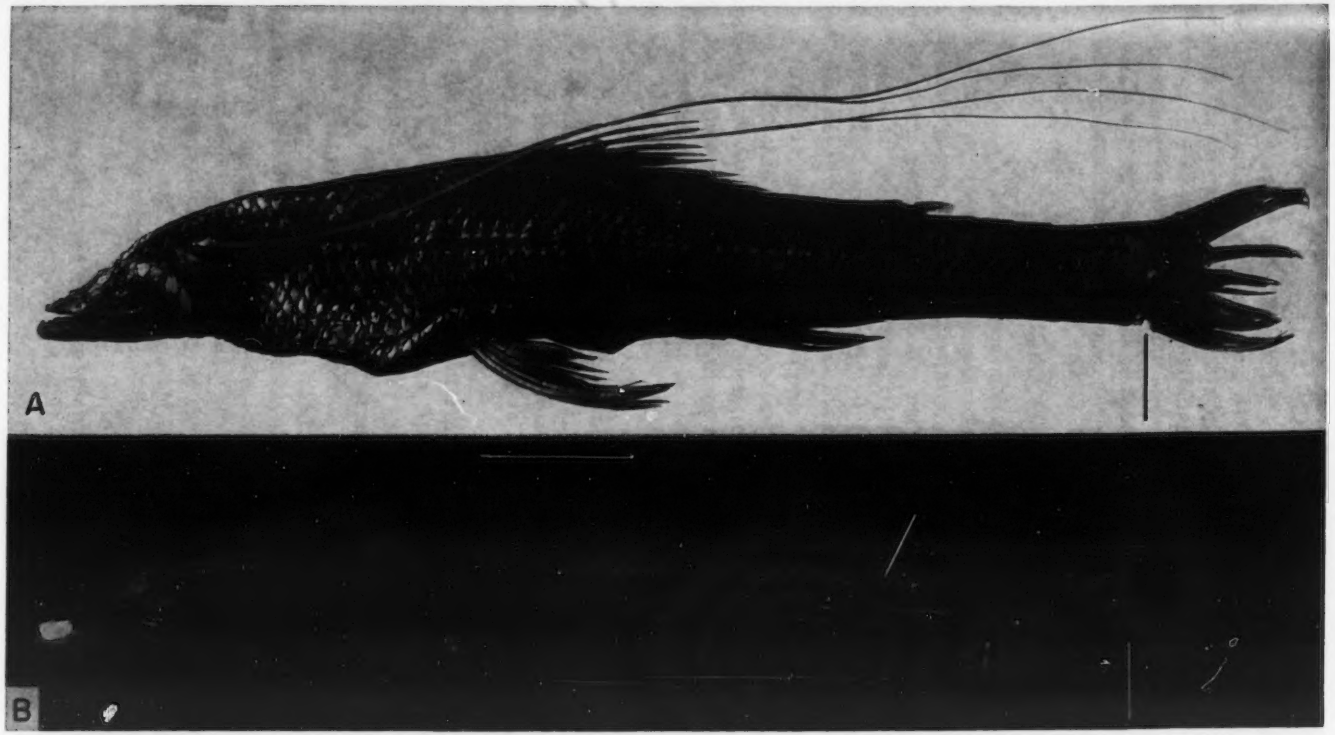

FIG. 1. *Bathypterois dubius*, 204 mm S.L., from the southwestern Grand Bank, May 2, 1959; **A**, photograph, **B**, X-ray picture of formalin specimen. The vertical lines indicate the notch at the base of the caudal which is typical of *B. dubius* and its relatives.

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dorsal approximately under beginning of the anal; the smaller number of lateral-line scales and vertebrae (ca. 54 and 54 in *B. phenax* against ca. 60 and 59 in the Grand Bank specimen); upper division of pectoral in *B. phenax* 2 + 1 to 2 + 4 (2 + 1 in the Grand Bank specimen); lower part of pectoral in *B. phenax* seven or eight rays (10 in the Grand Bank specimen). The latter, however, is not very significant since Mediterranean *B. dubius* have eight rays in the lower division of the pectoral.

COMPARISONS WITH *B. DUBIUS*

PECTORALS. The upper division of the pectoral in the Grand Bank specimen has the two upper fin rays combined at the base and a smaller free fin ray immediately below. The third fin ray and all 10 of the fin rays in the lower division of the pectoral were broken near the base. This third fin ray of the upper group is mentioned by Vaillant (1888) in his original description of the species and is shown in his figure (pl. 9, fig. 1, also copied in Goode and Bean (1896) but not in his pl. 9, fig. 1a or his pl. 12, fig. 4).

Collett (1896) says that the upper elongated ray of the upper division of the pectoral is comprised of two rays closely united and that below this ray is a short rudimentary ray. This latter ray is also shown in Collett's pl. 4, fig. 19. Zugmayer (1911) also mentions two detached rays of the upper part of the pectoral of which the first is elongated and divided and the second minute. Other authors (Koehler, 1896; Dieuzeide and Roland, 1957; Bougis and Ruivo, 1957; and Raimbault, 1963) do not mention this short ray but it appears to be present in Dieuzeide and Roland's figures. Garman (1899), for the closely related *B. pectoralis* of the Pacific Ocean, describes the upper portion of the pectoral as consisting of three rays (as in *B. dubius*), two of them very long and bound together in the greater portion of their length, and the third ray very small.

In the lower portion of the pectoral our specimen from the western Atlantic had 10 rays, those from the eastern Atlantic, 9–12, and all those from the Mediterranean eight rays (Table 1).

PELVICS. The pelvics in the Grand Bank specimen have the typical *B. dubius* form of two elongated external rays and six shorter rays. The length of the longest pelvic ray in the Grand Bank specimen is only 20.2% S.L. (Table II, Item 36) and the longest ray does not reach near the anal. In Vaillant's (1888) figure (pl. 9, fig. 1) the longest pelvic ray is about 43% of the S.L. and extends posterior to the anal fin. Vaillant, however, says that although for the individual taken as the type the elongated rays of the pelvic extend considerably beyond the anal, in other specimens they are much shorter and barely reach the origin of the anal. (See Vaillant's pl. 9, fig. 1c, and Bougis and Ruivo's range of 25.6–42.4% S.L. in seven of Vaillant's specimens, Table II.) These elongated rays of the pelvic range from 19.6% S.L. in one of Raimbault's specimens to 92.0% in that of Dieuzeide and Roland (Table II). Thus, the Grand Bank individual is included in the lower part of the range. In the

TABLE I. Meristic and other counts for a Grand Bank specimen of *Bathypterois dubius* and for others of this species from the eastern Atlantic and the Mediterranean.

No. of specimens included	Reference	Locality	Dorsal fin rays	Anal fin rays	Pectoral fin rays	Pelvic fin rays	Caudal fin rays	Scales in lateral line	Scales above lateral line	Scales below lateral line	Vertebrae	Branchiostegals	Gill rakers on anterior arch
1	Author	SW slope Grand Bank	15[a]	9[a]	2+1, 10	8	19[b]	57[c], 62[d]	6	8	59[e]	13	12+1+32
?	Vaillant (1888)	E Atlantic	15	9	2[f]+1, 10–12	8	19	60	6	8	58	12	...
3	Collett (1896)	E Atlantic	15–16	9	2[f]+1, 10–11	8	19	ca. 58–60	6	8	...	12	...
2	Koehler (1896)	E Atlantic	15	...	2[f], 10	8	...	...	6	7	...	...	...
3	Zugmayer (1911)	E Atlantic	14–16	9	2[f]+1, 10	8	20	58–60	6	8	...	...	...
2–11	Koefoed (1927)	E Atlantic	15	9	2, 9–11	8	...	ca. 58–60	...	...	...	...	...
1	Bougis and Ruivo (1957)	Mediterranean	16	9	2,8	8	19	...	...	...	...	12	11+29
1	Dieuzeide and Roland (1957)	Mediterranean	15	...	2, 8	8	...	...	...	...	...	...	...
4	Raimbault (1963)	Mediterranean	14–15	9–10	2, 8	8	...	56–58	...	...	...	...	...

[a]Counting all fin rays.
[b]All long rays, 10 above, nine below.
[c]Number to 1st scale touching shoulder girdle.
[d]Number to upper opening gill cavity.
[e]Including urostylar half vertebra as one vertebra.
[f]Upper combined two long rays of pectoral counted as one by these authors but as two in this table.

TABLE II. Body proportions of a Grand Bank specimen of *Bathypterois dubius* and of others of this species from the eastern Atlantic and the Mediterranean. (Unless otherwise indicated all measurements are percentages of the standard length. Standard lengths from chin tip instead of snout and percentages based on them are in parentheses.)

Body part	Author	Vaillant (1888)	Collett (1896)	Zugmayer (1911)	Koefoed (1927)	Bougis and Ruivo (1957)	Dieuzeide and Roland (1957)	Bougis and Ruivo (1957)	Raimbault (1963)
1. Specimens available, *No.*	1	70	3	2	11	...	1	1	4
2. Specimens included in measurements, *No.*	1	1	3	1	1	7	1	1	4
3. Locality of capture	SW slope Grand Bank	E Atlantic	E Atlantic	E Atlantic	E Atlantic	E Atlantic (Vaillant's *Talisman* specimens)	Mediterranean	Mediterranean	Mediterranean
4. Total length, *mm*	242+	243	237–ca. 256	180	193	...	210	...	ca. 147–230
5. Standard length, *mm*	204[a] (206)[b]	(185)[e,f]	(191–205)[e]	(155)[e]	(168)[e]	(127–203)[d]	113[e,g]	(87)[d]	(119–186)[d]
6. Head length, from snout, %	17.3[h] 18.3[i] (17.1)[h] (18.1)[i]	...	(18.5–20.4)	...	...	...	ca. 25	...	...
7. Head length, from ant. tip mandible, %	18.4[b] 19.3[i] (18.0)[h] (19.0)[i]	ca. 20.4[j] (24.3)	(21.0–22.0)	(20.0)	(20.5)	(19.3–21.0)	...	(21.3)	(21.9–23.5)
8. Snout length, ant. tip upper jaw to eye, %	6.1 (6.0)	ca. 5.6[j]	(5.9–6.8)	(5.8)	...	...	...	...	(5.9–6.3)
9. Snout length, from ant. tip of mandible to eye, %	7.0 (6.9)	(8.6)	...	...	(7.1)	(6.7–7.2)	...	(8.0)	...
10. Eye, horizontal diameter, %	1.5 (1.5)	ca. 1.6[j] (1.6)	...	(1.9)	...	(1.9–2.4)	...	(2.3)	(2.1–2.2)
11. Head, post-orbital length, %	11.4[i] (11.3)[i]	...	(11.7–12.0)	...	...	...	...	...	...
12. Interorbital space, %	7.0 (6.9)	(8.6)	...	...	(7.4)	(7.2–8.1)	...	(8.6)	(7.6–8.1)
13. Upper jaw, length snout to end maxillary, %	10.8 (10.7)	...	(11.7–13.1)	...	...	(11.0–13.0)	...	(12.6)	...
14. Mandible, length, %	12.4 (12.2)	...	(13.4–14.7)	...	...	...	...	...	(13.5–14.8)
15. Snout–origin dorsal, %	43.2 (42.8)	...	...	(41.9)	...	...	...	...	...
16. Ant. tip mandible–origin dorsal, %	44.1 (43.7)	...	(44.3–46.3)	...	(42.0)	(40.6–45.5)	...	(44.8)	(43.5–45.4)
17. Snout–origin pelvic, %	38.1 (37.8)	...	...	(38.7)	...	...	...	...	...
18. Ant. tip mandible–origin pelvic, %	39.2 (38.9)	...	...	...	...	(35.9–40.4)	...	(40.2)	...
19. Ant. tip mandible–anus, %	52.4 (51.8)	...	(52.7–53.9)	...	(49.4)	(50.3–54.2)	...	(56.3)	(51.3–55.1)
20. Ant. tip mandible–origin anal, %	63.0 (62.4)	...	...	...	...	(60.7–63.6)	...	(63.2)	...

(*continued*)

(*concluded*)

21.	Base last dorsal ray–base caudal, %	43.0[k] (42.6)[k]	...	...	...	(42.0)	...	...	...	...
22.	Base last dorsal ray–ant. base adipose, %	19.9 (19.7)	...	...	...	(17.9)	...	...	...	...
23.	Dorsal origin behind vertical ant. base pelvic, %	2.5	a little behind	...	...	...	...	yes (from fig.)	...	...
24.	Posterior insertion dorsal anterior to vertical from anterior insertion anal, %	4.0	ca. 5[j]	...	...	...	...	yes (from fig.)	...	...
25.	Greatest height, %	16.2 (16.0)	ca. 15.8[j] (16.8)	...	(15.5)	(12.2)	(10.3–14.3)	...	(16.1)	(16.0–16.8)
26.	Height body at insertion pelvics, %	16.2 (16.0)	...	(13.4–15.6)	...	...	...	...	...	...
27.	Height body at anus, %	15.3 (15.1)	...	(11.9–12.7)	...	...	...	...	...	...
28.	Caudal peduncle, least height, %	8.6 (8.5)	...	(7.3–7.8)	(11.0)	...	...	...	10.3	...
29.	Greatest width, %	9.7[i] (9.6)[l]	(10.8)	...	(7.7)	...	...	...	10.3	...
30.	Head width, %	9.6 (9.5)	...	(9.5–10.5)	...	...	...	...	...	...
31.	Dorsal, length longest ray, %	17.0 (16.8)	ca. 16[j]	(15.9–16.8)	...	...	...	...	...	...
32.	Anal, length longest ray, %	12.3 (12.1)	ca. 12[j]	(14.4–14.7)	...	...	...	...	...	...
33.	Caudal length, %	...	(31.4)	...	...	...	(23.2–29.1)	75.2	...	...
34.	Pectoral, total length uppermost combined rays, %	101.5 (100.5)	Beyond end of scales	(101.0)	(103.2)	...	(90.6(1))	97.3	(95.4)	...
35.	Pectoral, uppermost combined rays, length base to point of division, %	54.9	ca. 80[j]	...	...	...	...	...	...	...
36.	Pelvic, length longest ray, %	20.2 (20.0)	ca. 43[j]	(34.0–37.6)	...	...	(25.6–42.4)	92.0	(26.4)	(19.6–27.4)
37.	Dorsal, length base, %	17.0 (16.8)	ca. 14[j]	...	...	...	...	...	...	(16.4–18.3)
38.	Anal, length base, %	7.7 (7.7)	ca. 9[j]	...	...	...	...	...	...	(8.4–9.7)

[a]Snout to end hypural.
[b]Anterior tip mandible, with mouth closed, to end hypural.
[c]Probably ant. tip mandible to base caudal.
[d]Anterior tip mandible, with mouth closed, to base caudal.
[e]Snout to base caudal.
[f]Calculated from Vaillant's "body length" (which, judging from his description of body proportions on p. 124 and his table of body proportions on p. 132 is total length) less caudal length. The result probably is "ant. tip mandible to base caudal."
[g]From Dieuzeide and Roland's figure, natural size.
[h]To end bony operculum.
[i]To end opercular membrane.
[j]From pl. IX(1) Vaillant and S.L. snout to base caudal.
[k]To upper procurrent caudal rays.
[l]Not below lateral line.
[m]The following additional data on the Grand Bank specimen of *B. dubius*, are useful for comparison with Mead's (MS, 1954) descriptions of other western Atlantic Bathyteroidae (fork length in mm, remaining measurements as percentages of S.L. and other details as for Table II): total length to mid-fork, 230; snout–anus, 51.6 (51.1); snout–origin anal, 62.0; posterior insertion adipose – upper procurrent caudal rays, 22.8; base last anal ray – lower procurrent caudal rays, 29.1; posterior insertion pelvic – origin anal, 21.6; anus – posterior insertion pelvic, 11.8; anus – origin anal, 10.3.

closely related *Bathypterois pectoralis* of the Pacific Ocean there is great variation in the length of these outer pelvic rays. In some *B. pectoralis* these rays reach to the middle of the anal; in others they almost reach the base of the caudal and the ends of the two long rays are thickened and fleshy — possibly a sexual modification (Garman, 1899). In *B. dubius*, Vaillant (1888) and Collett (1896) consider them to be tactile organs.

OTHER BODY PROPORTIONS AND MERISTIC DATA. In body proportions and meristic and other body part numbers the Grand Bank specimen is within the same range as or close enough to the eastern Atlantic specimens of *B. dubius* to warrant acceptance as the same species (Tables I, II).

The head measurements and most measurements of the various parts of the head appear to be at or near the lower part of the range in the Grand Bank specimen.

The combination of a notch at the base of the lower caudal rays, the lack of a lower thick ray in the pectoral, the origin of the anal fin well behind the end of the dorsal (Fig. 1), and the possession of eight pelvic rays agrees with *B. dubius* (Mead, 1959, 1966) and with no other North Atlantic species.

Note added in proof. The gonads of this specimen were not sectioned and studied microscopically to determine whether in fact the "ovary" was an ovotestis (*see* Giles W. Mead. 1960. Hermaphroditism in Archibenthic and pelagic fishes of the order Iniomi. Deep Sea Res., **6**(3): 234–235) but no testicular material or male ducts could be seen in gross examination under the dissecting microscope.

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Bioassay Method for the Determination of Toxaphene in Lake Water[1,2]

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ABSTRACT

A method based on static bioassays was developed to measure the concentration of toxaphene before restocking of lakes treated with this toxicant. A double distillation technique was used to extract the toxaphene and concentrate it in a small volume of distillate. Bioassays were then carried out in 200-ml samples of distillate reconstituted with electrolytes to match a standard water. Guppies, *Lebistes reticulatus* (Peters), less than 7 days old were used as test organisms.

The method developed was used to follow the detoxification of Midway Lake which was rehabilitated with toxaphene. Data on the rate of detoxification are presented.

INTRODUCTION

In 1962, a study was initiated at the Saskatchewan Fisheries Laboratory to develop a suitable method which could be used to determine the quantity of toxaphene present in a sample of lake water. The long residual life of this chemical has resulted in many rehabilitated lakes retaining a low level of toxicity for extended periods of time and attempts to stock such waters have occasionally been unsuccessful because of delayed mortality.

Several avenues of approach are open to the biologist interested in the detection and measurement of pesticides in water. Bioassay techniques appear to be the easiest and most practical approach to the problem. They have been widely used to determine acute and chronic effects of toxicants and have proven satisfactory for determining levels of known residues. The caging of test fish in a lake may be useful for determining acute levels of toxicity, but is unsatisfactory for determining toxicity levels near the lethal threshold, when delayed mortality and difficulties in making repeated observations are encountered. The high cost of instruments required for chemical analyses precludes their use at many fishery laboratories.

A satisfactory method should include the following criteria: it should be accurate for concentrations as low as 0.25 ppb (μg/litre); water requirements should be relatively small; procedure should be easily followed by laboratory technicians; and, test organisms should be easily maintained under laboratory conditions. The availability of such a method would be an important step in overcoming problems associated with rehabilitation of lakes.

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[2]Contribution from the Fisheries Branch, Saskatchewan Department of Natural Resources, Prince Albert, Sask.

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PROCEDURE

A steam distillation technique was employed to extract the toxaphene from the original water sample and concentrate it in a small volume of distillate. Bioassays were then carried out in the distillate samples. This technique was used in an effort to reduce the time required for bioassay testing of low concentrations and thus minimize the loss of toxicant due to evaporation or absorption by fish and test containers.

Davidow and Sabatino (1954) employed a method of steam distillation to recover low concentrations of several insecticides, including toxaphene. They found that when a solution or material containing chlorinated hydrocarbon insecticides is boiled and the steam condensed, most of the toxicant from the original sample is found in the first 200 ml of distillate. Mount (1962), using a similar method, found that by collecting 250 ml of distillate from a 1000-ml solution known to contain 0.5 ppb endrin, it was possible to get a concentration of 1.5 ppb in the distillate, which represents a 75% recovery.

After experimenting with various volumes of solution and distillate, it was decided that collection of 200 ml of distillate by double distillation from an original sample of 10 litres gave satisfactory results with toxaphene. In the procedure used, a stock solution of technical grade toxaphene[3] was added to a 10-litre sample of water to give a solution of known concentration. This was equally divided and each 5-litre subsample was boiled in a 6-litre Florence flask connected to a Graham condenser. A 200-ml sample of distillate was collected from each 5-litre subsample and the two distillates were combined. The 400-ml sample of combined distillate was boiled a second time in a 500-ml flask and 200 ml of distillate was collected for fish bioassay.

Prior to bioassay, each distillate sample was reconstituted by the addition of 30 ppm calcium sulphate, 30 ppm magnesium sulphate, 48 ppm sodium bicarbonate, and 3 ppm potassium chloride, as recommended by Mr R. E. Lennon, Fish Control Laboratory, La Crosse, Wisconsin (personal communication).

Guppies, *Lebistes reticulatus* (Peters), were selected as test fish for bioassay experiments. This species is easily bred and maintained under laboratory conditions and proved to be relatively sensitive to toxaphene. Only guppies less than 7 days old were used to assay distillate samples. Mount (1962) found guppies at this age to be significantly more sensitive than adults to endrin, a chlorinated hydrocarbon insecticide similar to toxaphene. The small size of guppies at this age also permits the assaying of small volumes of test solution. In the present study three or four young guppies were assigned to each 200-ml distillate sample.

Bioassays of reconstituted samples were carried out in 1-quart glass jars in a water bath at 25 ± 1 C. Dissolved oxygen in the distillates ranged from 4.6 to 5.3 ppm prior to the introduction of test fish and usually increased to 6.4–6.7 ppm by the time the tests were completed. A dissolved oxygen content

[3]Donated by Hercules Powder Company, Wilmington 99, Delaware.

above 4.0 ppm is recommended by Doudoroff et al. (1951) when warm-water fish are used. The *p*H of the distillates, measured at the completion of each test, ranged from 7.6 to 8.0.

Samples of dechlorinated tap water and lake water were subjected to the distillation procedure and bioassayed as control experiments. Only one death occurred in the control groups (12 fish) within the time duration of the longest toxaphene bioassay.

The survival time of individual fish in each distillate was recorded and the mean survival time for 15 fish was determined for each concentration tested. Fish in the test solutions exhibited characteristic symptoms of chlorinated insecticide poisoning, i.e. hypersensitivity, erratic swimming, and finally loss of equilibrium. Test fish were considered dead when respiratory movement ceased and they failed to respond to a mild mechanical stimulation, such as prodding with a glass rod.

The results of distillate bioassays are shown in Fig. 1. Mean survival times for original toxaphene concentrations ranging from 0.25 to 5.0 ppb were plotted on a log–log graph. This represents a standard curve for toxaphene under the particular set of experimental conditions. The level of an unknown concentration can be determined by following the distillation procedure and comparing the mean survival time of 12–15 young guppies with the standard curve. If preliminary tests on an unknown sample indicate a concentration greater than 5.0 ppb, subsequent samples should be diluted with distilled water prior to distillation.

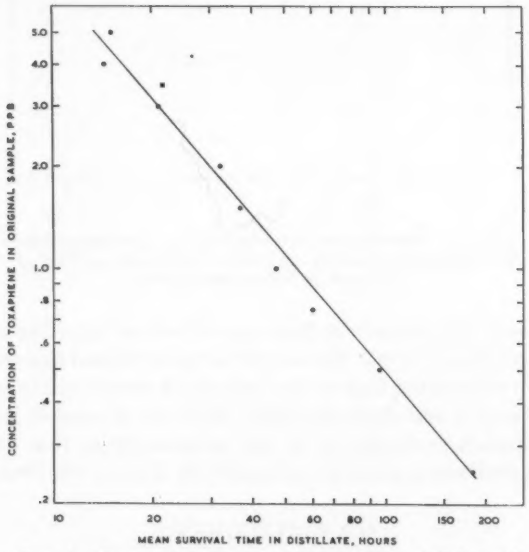

FIG. 1. Standard curve of survival values for 1–7-day-old guppies in 200 ml of distillate at 25 C (correlation coefficient for log concentration on log time, $r = -.852$).

APPLICATION

The bioassay method described in this report was used to follow the detoxification of Midway Lake (140 acres, max depth 15 ft), which was treated with toxaphene on September 9, 1964, at a concentration of 5.35 ppb. Water samples collected prior to the chemical treatment and at intervals following the treatment were analyzed by the distillation–bioassay technique.

The detoxification of Midway Lake is graphically shown in Fig. 2. The data illustrate the importance of temperature on the breakdown of toxaphene. A rapid rate of detoxification occurred between the time the lake was treated and freezeup (about November 10). Thereafter, while the lake was ice-covered, detoxification was apparently negligible. Mitchum (1963) in laboratory experiments showed that under various chemical and physical conditions, temperature was the only factor which significantly influenced the rate of toxaphene breakdown.

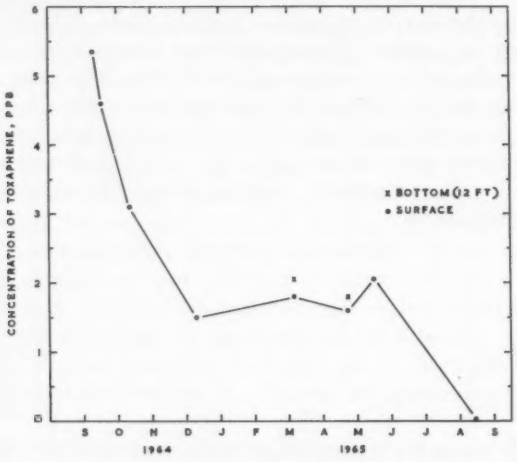

FIG. 2. Detoxification curve for Midway Lake determined by distillation–bioassay technique (toxaphene applied at 5.35 ppb on September 9, 1964).

An increase in the toxaphene level was observed after the spring breakup which occured on May 5, 1965. The source of the increased toxicity was presumably the bottom water since higher concentrations were noted in bottom samples collected on March 5 and April 22, 1965. With the advent of warmer temperatures, detoxification proceeded at a rate comparable to that observed during the fall of 1964 and was apparently complete by August 14, 1965.

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Change in Size-Class Structure of Populations of Kodiak Island Commercial Male King Crabs Due to Fishing[1]

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ABSTRACT

Intensive exploitation of commercial king crab populations at the south end of Kodiak Island has caused a reduction of individual crab size and, as the resource continues to be utilized at a high level, the recruits may eventually constitute the bulk of the harvest.

Carapace length distributions of 3359 males obtained on August 10–11, 1963, from three fishing areas with different rates of utilization indicated that the size-class structure for the Sitkinak Island and Wide Bay areas were similar, but different from that of the Alitak Bay area.

Comparison of length–frequency data collected from Alitak Bay in 1954 and 1963–64 indicated that larger size-classes have been cropped and are no longer abundant.

INTRODUCTION

Experiments were conducted aboard the king crab processing vessel *Mercator* at Kempff Bay during August 1963 to examine the commercial catch from three fisheries (Fig. 1) which have had different histories of utilization, and to determine the effects of exploitation within the populations. Samples were obtained from Wide Bay, the Sitkinak Island area, and the Alitak Bay area. Length–frequency and exoskeletal-age data were taken as the fishing boats unloaded their catches to the *Mercator*.

Since the king crab populations are dynamic, complex, and extremely variable this paper cannot evaluate the overall changes which may result from an altered environment. Hence, the report deals with only the most apparent bases of management to protect the brood stock and to crop at the critical size.

As large males are cropped by the fishery, smaller crabs invariably become the main brood stock. An unknown percentage of sublegal males are capable of reproduction (Powell and Nickerson, 1964), and if they are relied upon to act as brood stock the resulting annual increment is thought to be sufficient to maintain a substantial fishery, although the average size and weight would be reduced from its present level and fishing restrictions may have to be imposed in certain heavily exploited areas.

The authors assume that cropping the older age-classes, those 9 years and over, stimulates an increase in the population and rate of growth of its

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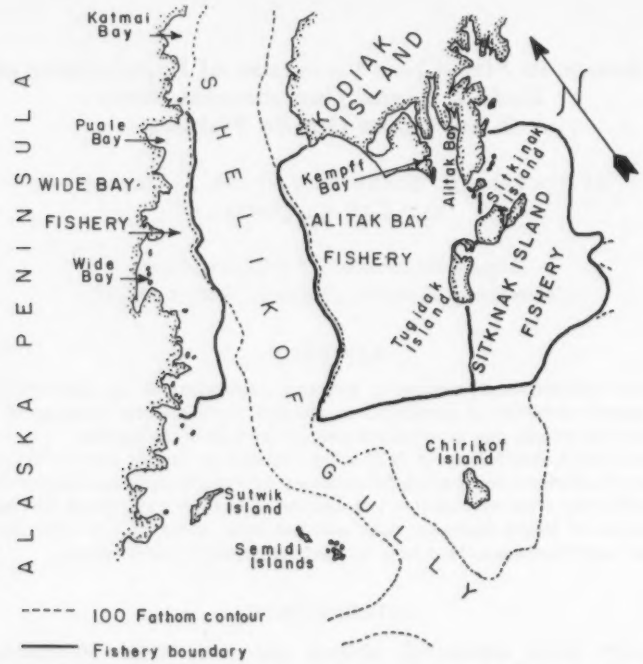

FIG. 1. King crab fishing areas at the south end of Kodiak Island, Alaska.

individual members (only males are cropped, and females are not dealt with in the paper). They also assume that (all other factors remaining constant): abundance of annual recruits may continue unchanged, or increase; reduced competition from the larger males would favor increased participation of the younger age-classes in breeding; survival would increase; average age at earliest anexuviation would be raised and, conversely, the proportion of anexuviants would be reduced.

Anexuviant males, those molting less frequently than annually, are present in all populations and we would like to determine whether their numbers vary with the intensity of exploitation. Some males begin biennial molting as early as their seventh year (Powell, 1964) when they are recruited into the commercial fishery. At this time they are approximately 145 mm in carapace length, which corresponds closely to the legal minimum carapace width of 178 mm. Triennial and quadrennial molting occurs among older individuals, usually commencing at the ninth year.

Frequency of molting is determined by the amount of wear and discoloration on the ventral surfaces of the coxopodites and meropodites, the tips of the dactylopodites, holding surfaces of the pollices and dactylopodites of the chelipeds, and by the amount and size of fouling organisms (i.e. barnacles,

bryozoans, turbellaria, etc.) on the carapaces of tagged crabs which were of a known exoskeletal-age when initially marked. Anexuviants may act as tags, though not as efficiently, by providing indices of age-class abundance and rates of utilization from which population trends can be analyzed.

Physiological aging, at present, is impossible unless crabs are tagged at a known age (Powell, 1964) of 6 years (approximately 120 mm carapace length) or less, and the same individuals are released anew after each recapture subsequent to the collection of pertinent data. From these data, trends in growth, exoskeletal aging, and anexuviation are applied in an appropriate manner to the unmarked population.

METHODS AND MATERIALS

Alitak Bay supports an intensive fishery, Sitkinak Island a moderate fishery, and Wide Bay less than moderate. Alitak is the oldest fishery, having been exploited since 1938. The Sitkinak Island fishery began in 1959 and the Wide Bay fishery in 1960, but the latter has been sporadic and underexploited due to unfavorable weather conditions and to more lucrative fishing grounds elsewhere.

Tag and recapture data (on file at the Kodiak Research Center, Kodiak, Alaska) reveal that there is apparently little intermingling among the crabs of Wide Bay and those of Alitak Bay, since only two Alitak tags have been recovered across Shelikof Strait, one in Katmai Bay and one in Puale Bay. A small percentage (0.6%) of 4301 Alitak tagged crabs have been captured over a 2 year period in the Sitkinak area, and a few Sitkinak tagged crabs recorded as captured in the Alitak fishery; but quantitative data concerning the latter are premature at this writing (Powell and Reynolds, 1965). Although the populations of the Alitak and Sitkinak areas appear delineated, both fisheries are augmented by migration of crabs from the vicinity of Chirikof Island and south of the Semidi Islands, but no marked crabs have been recovered from the Sutwik Island federal (U. S. Fish and Wildlife Service) tagging program of 1962, hence portions of the Sutwik Island stocks are assumed to move into the Wide Bay area (Powell and Reynolds, 1965). These data suggest that crabs on the mainland side of Shelikof Strait inhabit the bays and deep-water areas on the west side of the Shelikof gully, and crabs on the east side of the gully form a portion of the Kodiak Island stocks, but no conclusions can be drawn at this writing as to the number of crabs crossing the gully, and whether or not this movement is random or migratory.

Comparison of 1954 and 1963–64 length frequencies (Fig. 2) shows the relative degree of exploitation within the Alitak fishery over a 10 year period by the reduction of older age-classes. Seven 1954 samples (1466 crabs) were used for exoskeletal-age composition since exoskeletal-ages were recorded for only these samples. Twelve additional 1954 samples (1328 crabs) combined with the former seven show total size-class distribution. The 1954 samples were taken during March, April, and May from commercial catches with otter trawls (trawls were ruled illegal as king crab fishing gear in the Kodiak Island

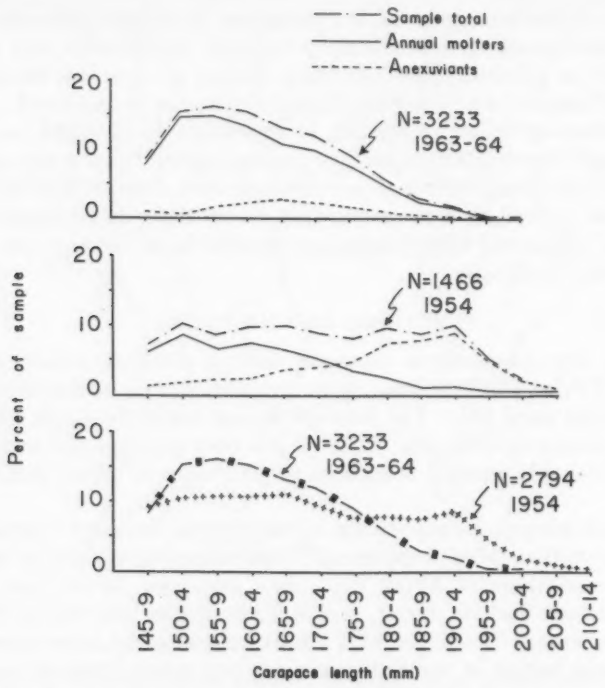

FIG. 2. Comparison of 1954 and 1963–64 length distributions and exoskeletal age compositions from Alitak Bay area.

district in 1960). During these 3 months king crabs occupy the breeding grounds and mating individuals are not readily attracted to baited pots, since feeding is usually suspended. The samples of 1963 and 1964 were taken during January, February, and September through December, pots being used as capture gear. Since trawls are more efficient during the breeding season and pots are superior at other seasons, sampling techniques and the results obtained on size-class distribution for the two sampling periods are considered to be comparable.

The proportion of anexuviant crabs 180 mm and larger in 1954 was reduced by 89% to the proportion obtained in 1963–64 (Fig. 2). Also shown in Fig 2 is the inverse relationship between anexuviants and annual molters, especially in the larger size-classes, that occurred from 1954 to 1963–64. Since larger crabs were fewer, the stock of recruits (145–164 mm) was utilized at an increase of 55% during 1963–64.

Although the 1963–64 Alitak length distributions in Fig. 2 and 3 are based on different samples, the curves are similar except for a 5 mm difference in the modes. In addition, the length distribution curve for Alitak in 1954

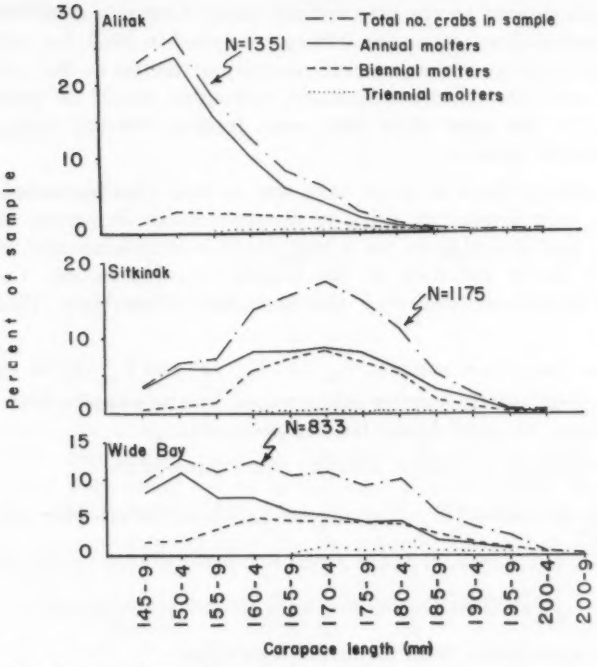

FIG. 3. Length distributions and exoskeletal-age compositions obtained August 10–11, 1963.

(Fig. 2) and that for Wide Bay in 1963 (Fig. 3) both show size-class structure profiles of relatively unexploited populations.

On August 10–11, 1963, 3359 king crabs of legal size were measured aboard the *Mercator*. The crabs were from the catches of five vessels, one from Wide Bay and two each from the Alitak Bay and Sitkinak Island areas. Crabs from the different areas were placed into separate live-holding tanks through which sea water was circulated, and held for a few hours prior to processing. The catch from each vessel was processed separately. After the tanks were drained, the crabs were individually pewed onto a conveyor belt which led to the butchers. To ensure a random sample crabs were removed unseen from the belt as they passed the measurer, where carapace lengths were measured. However, since 186 crabs were of sublegal size, they are not included in the following catch data or in the final analysis.

A major limitation of this investigation is not knowing how well the samples represented the three fishing areas. King crabs tend to shoal by exoskeletal-age and size-classes, which may differ according to individual growth rates and to the abundance of anexuviants in the various shoals. In addition, catch composition may vary when different shoals are in juxtaposition.

Analyses depend on the assumptions that: there was no differential selectivity or catchability among the fishing gear used in 1963 (i.e. pots); all king crabs in the vicinity of the gear were equally attracted to the bait; and since king crabs are intrinsically migratory, numerous shoals of them would be intercepted by the gear while they were feeding, thereby making a representative sample possible.

The main problem in these data was to test whether samples from the three areas were similar in size and exoskeletal-age frequency distributions. For this, it was desirable to use a test which is nonparametric and needs no assumptions about the form of the population distribution. Consequently, a modified Kolmogorov-Smirnov test was used (Rosenblatt, 1962; Conover, 1965).

The test procedure was: let $F_{1_{n_1}}(x)$, $F_{2_{n_2}}(x)$ and $F_{3_{n_3}}(x)$ be the empirical cumulative distribution functions determined by the samples from Wide Bay, Sitkinak Island area and Alitak Bay, respectively.

The Kolmogorov-Smirnov statistic was expressed as

$$D_{n_i, n_j} = \sup_x \left[F_{i_{n_i}}(x) - F_{j_{n_j}}(x) \right], \text{ (Fisz, 1963, p. 445–449)}$$

where, $i = 1, 2, 3; j = 1, 2, 3; i \neq j$ and $\sup_x$ is the greatest difference between $F_{i_{n_i}}(x)$ and $F_{j_{n_j}}(x)$. The D_{n_i, n_j} was used to test the hypothesis that $F_{i_{n_i}}(x)$ and $F_{j_{n_j}}(x)$ were drawn from the same population.

The critical region used is to reject the hypothesis that $F_{i_{n_i}}(x) \equiv F_{j_{n_j}}(x)$ if $D_{n_i, n_j} > K$ where K is obtained from tabulated values for various α levels of significance (Birnbaum and Hall, 1959).

RESULTS AND DISCUSSION

Catch composition of the crabs measured aboard the *Mercator* from the three areas is shown in the following table. King crabs ranging in length from 145 to 164 mm represent the recruitment. Biennial and triennial molters within the recruit size-range had been available to the fishery for 1 and 2 years, respectively, prior to their capture.

	Alitak Bay	*Sitkinak Island*	*Wide Bay*
Number of boats	2	2	1
Total sampled	1351	1175	833
Depth of capture (*fath*)	75	50	120
Average length (*mm*)	153.5	167.5	165.0

Percentage molted			
Recently	85	60	55
Biennially	14	39	41
Triennially	1	1	4
Molters as percentage of recruits			
Recent	89	73	74
Biennial	10	26	26
Triennial	1	1	0

The samples from the two boats which were fishing in the Alitak Bay area were tested for a significant difference using the Kolmogorov-Smirnov statistic (Miller and Kahn, 1962, p. 464–470). Testing at the .05 level of significance they were found not to be different. Similar results were obtained by testing the samples of the two boats from the Sitkinak Island area. The length distribution of samples from the three areas is shown in Fig. 3.

The test results are:

Area tested	Symbol	D_{n_i}, n_j	K	Conclusion at $\alpha = .05$
Wide Bay vs. Alitak Bay	$F_{1_{n_1}}(x)$ vs. $F_{3_{n_3}}(x)$	0.348	0.275	$F_{1_{n_1}}(x) \not\equiv F_{3_{n_3}}(x)$
Wide Bay vs. Sitkinak Island	$F_{1_{n_1}}(x)$ vs. $F_{2_{n_2}}(x)$	0.166	0.285	$F_{1_{n_1}}(x) \equiv F_{2_{n_2}}(x)$
Sitkinak Island vs. Alitak Bay	$F_{2_{n_2}}(x)$ vs. $F_{3_{n_3}}(x)$	0.508	0.265	$F_{2_{n_2}}(x) \not\equiv F_{3_{n_3}}(x)$

These results show that the Alitak Bay sample differed significantly from the Wide Bay and Sitkinak Island samples, but the Wide Bay and Sitkinak Island samples were not significantly different.

The frequency distribution of biennial molters was normal in form for the Wide Bay and Sitkinak Island fisheries (Fig. 3) and this group composed 43 and 40%, respectively, of the catches. Biennial molters composed only 16% of the catch in the Alitak Bay fishery. Evidently, the Alitak Bay fishery quickly crops the crabs which are over 150 mm in length, and the males taken in this intense fishery must be smaller than those in a natural or not as intensely exploited population.

ACKNOWLEDGMENTS

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Cause and Control of Spurious Thiobarbituric Acid Values from Fish Muscle in the Presence of Inorganic Iron Salts[1,2]

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ABSTRACT

The distillation method for determining the thiobarbituric acid value gave spurious results when applied to cod muscle containing trace amounts of Fe^{+++} or Fe^{++}. This was the result of metal-catalyzed oxidation that took place in the acidified tissue during distillation. It was prevented by replacing the oxygen in the distillation flask with nitrogen or by immobilizing the iron catalyst by addition of the disodium salt of ethylenediaminetetraacetic acid or propyl gallate.

Complex organic iron compounds, copper salts, cysteine, or sodium chloride did not interfere with the TBA test.

INTRODUCTION

In a previous study of copper-induced rancidity in fish muscle (MacLean and Castell, 1964), a good correlation was found between the thiobarbituric acid (TBA) test (Tarladgis et al., 1960) and organoleptic ratings. Attempts to find a similar relationship between TBA values and the rancid odours developing in the muscle of cod following the addition of very small amounts of inorganic iron salts gave results that were perplexing. Although high TBA values were obtained from samples that were organoleptically rancid, almost equally high values were obtained from fresh fish muscle to which 5–20 ppm Fe^{++} had been added but which had no trace of rancid odour. Irrespective of the condition of the fish muscle, the mere presence of iron salts produced TBA values that are usually indicative of advanced rancidity. For this reason the TBA test, using the distillation procedure, cannot be used as a satisfactory measure of rancidity in fish muscle where the oxidation has been catalyzed by the addition of trace amounts of inorganic iron salts.

This is not the first occasion where inorganic iron salts have presented problems in the use of the TBA test as a measure of oxidative rancidity. Bernheim et al. (1948), without any elaboration simply stated that experiments with inorganic iron and copper as catalysts were inconclusive because they interfered with the TBA test.

Malonaldehyde is the principal compound in oxidized lipids that reacts with TBA to give the red pigment on which the test is based (Patton and Kurtz, 1951; Sinnhuber and Yu, 1958; Yu and Sinnhuber, 1954). This same aldehyde also reacts with ferric chloride to give a compound having a red

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[2]This is No. 6 in the series of papers dealing with "Rancidity in Lean Fish Muscle."

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colour (Claisen, 1903; Patton et al., 1951; Kwan and Watts, 1963). In fact, this reaction is the basis for a very sensitive test for malonaldehyde (Saslaw and Waravdekar, 1965). This complicates the TBA test for rancidity in those procedures where the TBA is added directly to a fatty substrate that contains both malonaldehyde and ferric ions. Jacobson et al. (1964), in a modified TBA test using a single phase solvent, also found that ferric chloride caused some interference. They suggested that the ferric chloride caused a breakdown of the TBA because of the high readings for the blanks when it was present.

In the procedure used for carrying out the TBA test in experiments with fish muscle the TBA-reactive substances are separated from the substrate by distillation before the TBA reagent is added, and presumably any inorganic iron present will remain behind in the residue. Under these conditions one would expect that if iron was going to cause trouble it would be during the distillation rather than during the reaction with the TBA reagent.

The purpose of this study was to investigate this interference of inorganic iron with the TBA test, to determine the conditions under which it occurs and, if possible, to find a method for preventing it.

GENERAL PROCEDURES

The procedure used for the TBA test was that given by Tarladgis et al. (1960), and its application to the study of rancidity in lean fish muscle has been described in detail in a previous paper in this series (MacLean and Castell, 1964). In brief, it is as follows: whole, fresh, cod fillets were blended with twice their weight of distilled water. Seventy-five-gram portions were weighed into 250 ml Erlenmyer flasks. Twenty-five milliliters of the solution containing the metal salts, or other reactants, were added to the homogenate and mixed by vigorous rotary motion. A separate flask was prepared for each test. The flasks were corked and immediately stored at 0 C for the desired length of time. For the TBA test 40 g of this homogenate was mixed with distilled water to bring the final volume to 110 ml. It was acidified to *p*H 1.5 by adding approximately 2 ml of 4 N HCl. The mixture was then distilled at a rate to give a total of 50 ml of distillate in 10–12 min. Five milliliters of the distillate was combined with 5 ml of 0.02 M TBA solution, in 90% acetic acid, heated in a stoppered tube in boiling water for 35 min and then cooled in running tap water. The optical density of the solution was read in a spectrophotometer at 538 mμ. The term "TBA value" is used to indicate the optical density reading after reaction with the thiobarbituric acid. The chemicals used in preparing the TBA reagent and for carrying out the test were purified as recommended by Sinnhuber and Yu (1958).

Tarladgis et al. (1960) found that when the *p*H of the material in the distillation flask was reduced below a certain level, and when the heating was prolonged, additional oxidation took place, giving false TBA values. This occurred at *p*H 0.5 and 30 min heating. At *p*H 1.5 and 10 min heating no significant oxidation took place during the distillation. Under these latter

conditions the test could be used to give approximately zero reading with fresh meat that had not been allowed to develop any rancidity. However, the apparent absence of an induction period in cod muscle to which Fe^{++} had been added suggested that rapid oxidation of the lipids might still be taking place during distillation. Iron will not catalyse the oxidation of unsaturated fatty acids in the absence of oxygen. Our first experiments, therefore, were carried out to see whether the spurious TBA values could be eliminated by replacing oxygen in the blended muscle with nitrogen before the distillation was carried out. This was done by evacuating the flasks several times and flushing them with nitrogen before the Fe^{++} was added. The addition of the solution containing the metal and the distillation itself were both carried out in a manner to prevent atmospheric oxygen from diffusing into the blended muscle. The distillates, however, were not protected from the atmosphere.

Once it was found that rapid oxidation was taking place during distillation, further tests were carried out to see whether evacuation and the addition of nitrogen could be replaced by antioxidants or compounds that would chelate or precipitate the iron. Finally, tests were made to determine whether organic iron compounds, Cu^{++} and other prooxidants, when added to the fish muscle, would cause spurious TBA values, in a manner similar to the inorganic iron salts.

EFFECTS OF REPLACING OXYGEN WITH NITROGEN

Fresh, blended cod muscle, acidified and prepared for distillation in the usual manner, was divided into two portions. Twenty parts per million Fe^{++} was added to the first portion and then distilled in the ordinary manner. In the second portion the oxygen was replaced with nitrogen before adding the Fe^{++}, and it was distilled with precautions taken to prevent contamination with atmospheric oxygen. Distillates from these two differently treated portions gave the following mean TBA values in five tests with different samples of cod muscle: without nitrogen, 0.344; with nitrogen, 0.059. A similar series of tests using both ferric and ferrous chlorides and sulphates, with nonrancid muscle, resulted in high TBA values by the ordinary procedure, and almost completely suppressed oxidation when nitrogen was used.

The preceding tests were all made with muscle that was not rancid. As the rate of autoxidation of fatty acids tends to increase with the degree of oxidation (that has already developed), further TBA tests were made on muscle in various stages of iron-induced rancidity (Fig. 1). Fe^{++} was added to blended cod muscle and then stored at 0 C. The rapid development of rancid off-odours indicated that the Fe^{++} rapidly accelerates rancidity in cod muscle. Within a few minutes after the iron had been added the first off-odours could be observed, and within the hour strong, fishy-seaweedy odours were present. (In other tests it had been observed frequently that within an hour after adding Fe^{++} to the muscle the rancid odours were as strong as those developing 15–20 hr after adding an equivalent concentration of Cu^{++}.) The relation

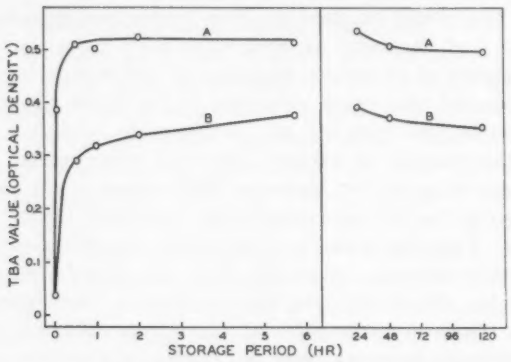

FIG. 1. Effect of replacing oxygen in the distillation flasks with nitrogen on the TBA values from blended cod muscle containing 20 ppm Fe^{++} and stored for various periods at 0 C. A — values with oxygen present B — values with oxygen replaced by nitrogen.

between TBA values and the development of rancid odours was much closer to those obtained from previous findings with rancid fish muscle using Cu^{++} as the catalyst (MacLean and Castell, 1964).

Solutions containing $FeCl_3$, $Fe_2(SO_4)_3$, $FeCl_2$, and $FeSO_4$ were made up to give concentrations of 20 ppm of the metal ions. These were acidified, distilled, and the distillates were tested with the TBA reagent in the usual manner. As might be expected, in the absence of the muscle tissues, they all gave negative readings.

EFFECTS OF ANTIOXIDANTS AND CHELATING AGENTS

A possible alternative method to removing the oxygen from the distillation flask was in the use of some compound that would remove or inactivate the iron. The very low *p*H, 1.5, and the boiling temperatures in the distillation flask did not favour the use of antioxidants or chelating compounds. However, initial tests with a number of these reagents gave results that showed considerable promise (Table I). As this is a much simpler and more convenient procedure, it was decided to explore this field further using the disodium salt of ethylenediaminetetraacetic acid (EDTA) and propyl gallate (PG).

The first experiments in this series were again carried out with fresh, nonrancid muscle to which Fe^{++} was added before distillation. The object was to find out the concentrations of PG and EDTA, when added to the blended muscle, that would be required to suppress oxidation in the presence of given concentrations of Fe^{++}. Figures 2, 3, and results of similar tests showed that when the fish was not rancid to begin with and when the concentration of EDTA or PG was more than 10 times that of the added Fe^{++} the oxidation during distillation was almost completely suppressed.

TABLE I. Effect of antioxidants and chelating agents, added immediately before distillation, on the TBA values obtained from fresh cod muscle containing 1 ppm added Fe^{++}.

Added compounds (to give 0.1%)	TBA value
Control — without added Fe^{++}	0.020
Control — with 1 ppm added Fe^{++}	0.175
Cysteine	0.235
Ascorbic acid	0.180
Potassium oxalate	0.176
Butylated hydroxytoluene	0.170
Citric acid	0.150
Cupferron	0.080
Polyphosphate	0.071
Nordihydroguaiaretic acid	0.065
Disodium ethylenediaminetetraacetate	0.040
Propyl gallate	0.025

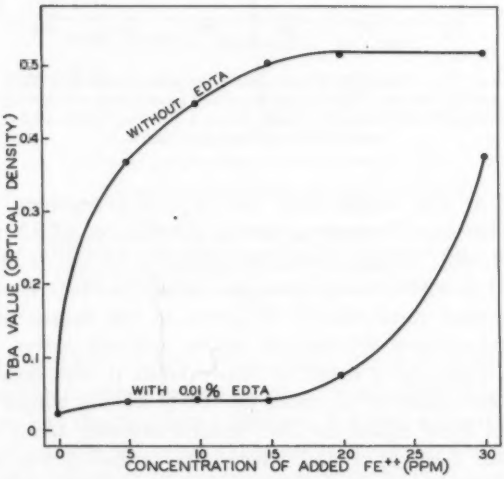

FIG. 2. The effect of adding 0.01% EDTA on the TBA values of fresh, nonrancid blended cod muscle containing from 0 to 30 ppm added Fe^{++}. The iron and EDTA were both added immediately before distillation.

In these tests there was a very short time lapse between the addition of Fe^{++} and the addition of PG or EDTA. The results were quite different if the iron-induced rancidity was allowed to develop before an attempt was made to measure the TBA value. It then required much greater concentrations to stop oxidation during distillation (Fig. 4). Comparisons were also made of the relative effectiveness of replacing the oxygen by nitrogen with removal

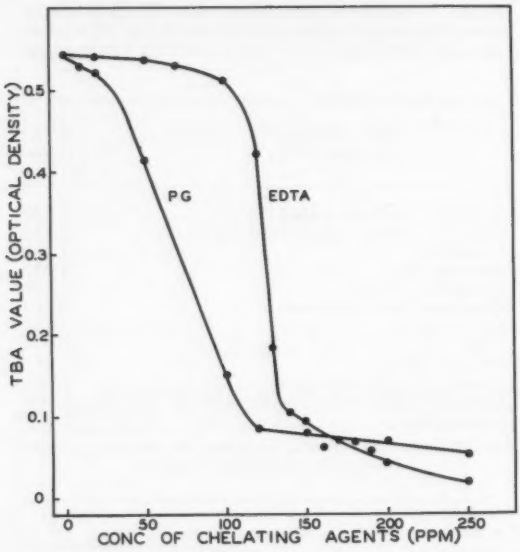

FIG. 3. The effect of increasing concentrations of EDTA and PG on the TBA values obtained from fresh, nonrancid blended cod muscle to which 20 ppm Fe^{++} had been added immediately before distillation.

of iron by EDTA. The results from one typical experiment indicated them to be equally effective: Treatment during distillation (TBA value in parentheses): none (0.460); oxygen removed nitrogen added (0.290); 1% EDTA added (0.280); 1% EDTA and nitrogen added (0.295). However, in using EDTA, consideration must always be given to the degree of rancidity and the concentration of iron the muscle before one can arrive at the amount of EDTA to be used. As a result of many trials it was concluded that the nitrogen treatment could be replaced by 0.1% EDTA where not more than 5 ppm Fe^{++} had been added to the blended muscle; and 1–2% where the Fe^{++} content was 5–20 ppm and where the rancidity was well advanced. PG was equally satisfactory in the same concentrations. Mixtures of equal amounts of 0.5% EDTA and 0.5% PG consistently gave slightly better values than 1% of either one alone. When using more than 0.5% of these antioxidants, the results were equally effective when they were added to the blended muscle as an aqueous solution or poured in as a powder and dissolved by gentle agitation.

The following comparison (four tests) shows the reproducibility of results and the effectiveness of nitrogen and the two antioxidants in supressing the unwanted oxidation when 20 ppm inorganic iron was present in the blended muscle: Treatment during distillation (mean TBA value $\pm$ standard deviation): none ($0.549 \pm .052$); nitrogen ($0.351 \pm .008$); 1% EDTA ($0.351 \pm .002$);

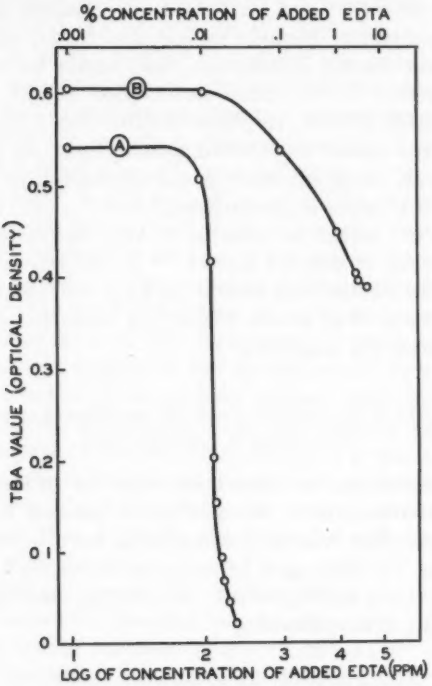

FIG. 4. Effect of increasing concentrations of EDTA (in the distillation flasks) on the apparent TBA values obtained from (A) nonrancid and (B) rancid blended cod muscle containing 20 ppm Fe^{++}.

1% PG ($0.315 \pm .015$); 0.5% PG + 0.5% EDTA ($0.305 \pm .003$). These results were similar to other tests that were made. EDTA and nitrogen were equally effective but the nitrogen treatment requires a great deal more attention to details in order to get results that are consistently the same from similar samples. The PG, alone or in conjunction with EDTA, gave slightly lower values. However, for all practical purposes all the TBA values for the treated samples are of approximately the same order.

COMPARISON OF Fe^{++} AND Fe^{+++} SALTS

All the preceding tests were made with Fe^{++} rather than Fe^{+++} because it had been found that Fe^{++} was much the stronger catalyst of rancidity in fish muscle stored at 0 C. But during the distillation, when neither nitrogen nor an antioxidant was added, Fe^{+++} always increased the TBA values more than Fe^{++}. These differences in the activity of Fe^{+++} and Fe^{++} under the two different conditions are illustrated in the following experiments.

When 20 ppm of Fe^{+++} and Fe^{++}, both as chlorides and sulphates, were added to similar samples of blended muscle and tested immediately without controlling oxidation during distillation, the results were as follows (TBA values in parentheses): Fe^{+++} added as chloride (0.333), sulphate (0.310), Fe^{++} added as chloride (0.150), sulphate (0.270). When this same concentration of iron salts was added to blended muscle, held for an hour, and then tested for TBA value, using nitrogen to control oxidation during distillation, the results were (TBA values in parentheses): Fe^{+++} added as chloride (0.170), sulphate (0.110), Fe^{++} added as chloride (0.440), sulphate (0.415). It would appear, therefore, that at *p*H 1.5 the Fe^{+++} is the stronger catalyst and at the normal *p*H of the blended fish muscle (*p*H 6.2–6.9) the Fe^{++} is the stronger catalyst. Or it could be that at the higher *p*H there is less Fe^{+++} available, because of the difference in solubility.

EFFECT OF ORGANIC IRON, COPPER SALTS, AND OTHER COMPOUNDS

Tests were carried out to determine whether prooxidants other than Fe^{+++} and Fe^{++} interfered with the distillation method for measuring TBA values in cod muscle. The following compounds were tested and were found to be noninterfering: 50–1000 ppm hemoglobin, myoglobin, hemin, and catalase; 5–50 ppm Cu^{++} (as $CuSO_4$, $CuCl_2$, $Cu(NO_3)_2$, and $Cu(CH_3CO_2)_2$; 0.5% cysteine and 1–10% sodium chloride.

SUMMARY AND DISCUSSION

The distillation method of Tarladgis et al. (1960) for determining TBA values is particularly useful for measuring oxidative rancidity in cod muscle and other tissues containing polyunsaturated fatty acids in the form of phospholipids and lipoproteins. Because it can be applied directly to the tissues without prior extraction with fat solvents it eliminates errors due to oxidation during extraction and handling. Tarladgis et al. also suggest this procedure would be expected to measure oxidation products of protein-bound lipids and phospholipids not extracted by ordinary fat solvents.

However, without some modification this procedure cannot be used to measure rancidity in tissues containing as little as 0.1 ppm Fe^{+++} or Fe^{++}. Irrespective of the actual extent of lipid oxidation the mere presence of these metal ions results in TBA values that are usually indicative of advanced rancidity.

It has been shown that the error is caused by iron-accelerated oxidation taking place during distillation in preparation for reaction with the TBA reagent. It has also been shown that this unwanted oxidation can be suppressed by evacuating the air from the distillation flask and replacing it with nitrogen. It can also be suppressed by precipitating the iron with PG or chelating it with EDTA. The concentration of PG or EDTA necessary to completely

suppress the oxidation is greatly increased by a time lapse between the addition of the iron salt and the addition of the antioxidant. Although the concentration of EDTA or PG needed to remove the iron ions will vary with the amount of iron present in the muscle (and also with the degree of rancidity at the time of testing), it was found that two general recommendations gave satisfactory results: If the muscle contained less than 5 ppm Fe^{+++} or Fe^{++} the addition of 0.1% EDTA or PG was sufficient. If the muscle contained 5–20 ppm Fe^{+++} or Fe^{++}, 1–2% should be used. The experimental data (and hence recommendations for control) dealt only with muscle with iron ion concentrations in the range of 0–20 ppm. In excess of 20 ppm of these metal ions, it is advisable to rely on the complete removal of oxygen from the distillation flask rather than on the chelating or precipitating agents.

In testing for rancidity in this laboratory, if there is any doubt as to whether inorganic iron is present in fish muscle, meat, or any other food product, our present practice is to exclude oxygen from the distillation flask or add a 1% mixture of EDTA and PG. The presence of these compounds in the distillation has never been found to interfere with the TBA test. However, we do not routinely use these precautions in measuring rancidity in lean fish muscle unless we have added inorganic iron or suspect it to be present. Our experience has shown that normally there are not sufficient iron ions in the muscle to interfere with the test.

It was observed that although the addition of Fe^{++} to muscle at *p*H 6.2–6.9 and stored at 0 C invariably resulted in a much greater development of rancidity than from a similar addition of Fe^{+++}, the Fe^{+++} is the more active catalyst under the conditions prevailing in the distillation flask (i.e. *p*H 1.5 and higher temperatures).

It has also been shown that these spurious TBA values, obtained when Fe^{++} or Fe^{+++} has been added to the muscle, do not occur when organic iron compounds, copper salts, cysteine, or sodium chloride have been used to induce rancidity.

Since this work was completed, McKnight and Hunter (1965) reported similar conclusions regarding the effect of inorganic iron on the TBA reaction. The procedure they used was to add acidified TBA reagent directly to diluted suspensions of the oxidized material, heat for 10 min at 100 C and then read the absorbancy at 535 mμ. When inorganic iron was present during heating of the acidified mixture, abnormally high TBA values resulted. This could be avoided by carrying out the reaction under anaerobic conditions. Essentially, however, the trouble was traced to the very active catalytic effect of iron combined with increased temperature and low *p*H.

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Number of Circuli and Time of Annulus Formation on Scales of Pink Salmon (*Oncorhynchus gorbuscha*)[1]

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ABSTRACT

Mean numbers of circuli on scales of juvenile pink salmon collected along the coast of North America in 1964 increased from 1.7 on June 23 to 17.4 on September 10. On scales of adults collected from the eastern North Pacific Ocean from 1962 to 1965, mean numbers of circuli ranged from 24.1 on January 23 (1964) to 46.9 on September 2 (1962). The fork length at time of scale formation was approximately 60 mm. Mean fork length increased with the total number of circuli. The relationship of fork length to number of scale circuli showed some variation between stocks. The winter ring was present or was being formed on the majority of scales taken in late January. The development of the annulus varied considerably among individual fish.

INTRODUCTION

This paper presents data on the number of circuli and time of annulus formation on pink salmon scales taken from fish sampled along the coast of North America and from the eastern North Pacific Ocean. Samples of juvenile pink salmon were collected in 1964 from June 23 to September 10. Adult fish were collected from January 22 to September 2 on different dates in the years 1962–65. The samples were not examined by sex; it was assumed that the number of scale circuli does not differ with sex. This assumption was based on the findings of Enyutina (1963) with mature Asian pink salmon.

Circuli are ridges on the surface of fish scales. Under microscopic examination, pink salmon circuli appear as dark rings circling a point near the center of the scale. Circulus widths and distances between circuli vary. Groups of thin circuli lying close together are known as "checks" or "rings." The most common is a check found on scales after a year's growth — the annual ring or winter ring (Fig. 1). The annulus is the last narrow circulus of the winter ring.

The circuli and annulus are of interest in growth and racial studies of North American pink salmon. Little information, however, has been published on the circulus formation or on the time the annulus is laid down.

Pink salmon are taken in the North American commercial fisheries from Puget Sound, Washington, to Bristol Bay, Alaska. Adults enter the coastal streams in the fall and die soon after spawning. Their young migrate to sea the following April, May, and early June shortly after they emerge from the gravel bottom of the river. They spend the following year in the North Pacific

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Ocean or Bering Sea and then return to their natal stream to spawn at an age of 2 years.

EXPERIMENTAL PROCEDURES

Sampling

The study is based on seven samples of juvenile pink salmon containing 607 specimens, and 16 samples of adult pink salmon totaling 689 fish. Locations and dates of capture are shown in Fig. 2.

The juvenile fish were collected from June 23 to September 10, 1964. The samples of June 23, July 13, and August 1 were taken from a small boat with a hand-operated seine. A large purse seine with a small mesh bunt was used to capture the fish of August 14, August 31, and September 10. The sample of July 9 was taken with a trawl-type net towed near the surface by two vessels.

The adult fish were collected from January 22 to September 2, 1962–65. The samples of January 22, January 23, April 10, April 25, April 28, May 19, May 30, and June 25 were taken with Japanese longline salmon gear. A large purse seine was used to capture the fish of June 14, July 12, July 23, and July 26. The remaining samples were caught by Bureau of Commercial Fisheries research vessels by gill nets with mesh sizes of $2\frac{1}{2}$, $3\frac{1}{4}$, $4\frac{1}{2}$, and $5\frac{1}{4}$ inches, stretched measure.

Scale Selection and Measurement of Body Length

Since variation of scale characters with respect to position on the body has been observed by Hayashi (1957), all scales were taken from a standard location on the side of the fish to minimize variability in circulus counts. For both juveniles and adults, the "preferred" scale was from a position two rows above the lateral line on the diagonal scale row which extends downward and posteriorly from the posterior insertion of the dorsal fin on either the left or right side of the fish. If both of these scales were missing or regenerated, the scale was taken slightly anterior or posterior to the "preferred-scale" position. This area included the first four scale rows above the lateral line and extended from a point directly below the posterior insertion of the dorsal fin to a point midway to the adipose fin.

Scales from adult fish were cleaned by rubbing between the fingers to remove the attached skin and were mounted with the sculptured side up on gummed paper. Plastic impressions of the mounted scales were made on clear cellulose acetate cards (Clutter and Whitesel, 1956). Scales from the juvenile specimens were scraped from the preferred body area with a scalpel and were mounted on glass microscope slides. The juvenile scale with the larger number of circuli was selected for use. A projection microscope was used to examine all scales at 100 × magnification (Mosher, 1950).

Measurements of fork length (distance from the fork of the tail to the tip of the snout) were taken from fish preserved in 10% formalin solution,

FIG. 1. Scale from an adult pink salmon showing the winter ring (a) and focus (b).

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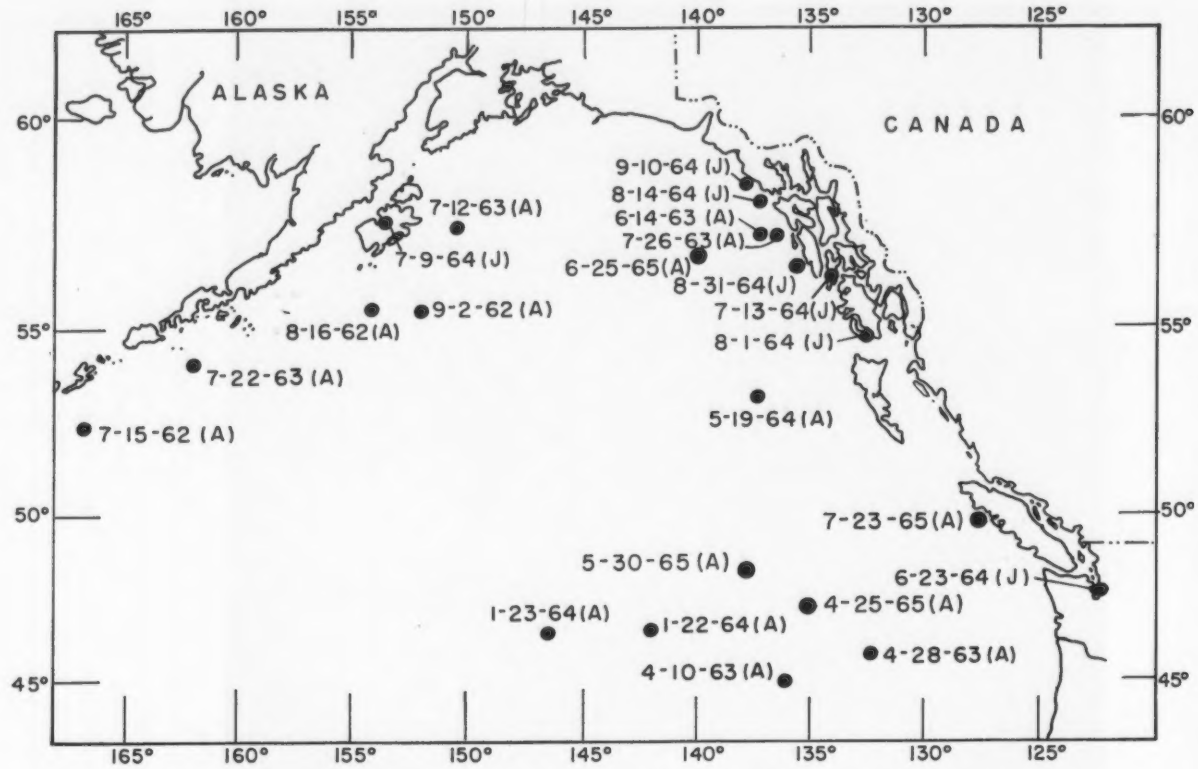

FIG. 2. Sampling locations and dates of capture of juvenile (J) and adult (A) pink salmon.

from fresh dead specimens, and from anesthetized live fish. Length measurements taken from specimens preserved in formalin (samples of June 23, July 9, July 13, and August 1) were increased 5% to compensate for shrinkage (Shetter, 1936; Parker, 1963).

Selection of Scale Axis

The circuli on pink salmon scales form concentrically around a central platelet or focus (Fig. 1) on the imbedded portion of the scale. Although circulus counts can be taken along any axis from the focus, they vary with choice of radius (Pearson, 1964). To minimize variability among scales, all circuli were counted from specific radial lines. Two methods of selecting a scale axis were used in this study — one when scales had less than 16 circuli and another when scales had 16 or more circuli.

The method for scales with fewer than 16 circuli was as follows: (1) one end of a pair of dividers was placed in the center of the focus, (2) the most distant point on the periphery of the last circulus (the greatest distance from the center of the focus) was determined with the dividers, and (3) the line formed by the two points was the scale axis.

When scales have 16 or more circuli, the greater number of broken or branched circuli may make it more difficult to select the same axis repeatedly. Although the previously described method was faster, the following procedure gave more consistent results (Pearson, 1964): (1) the longest axis to the annulus was selected visually, (2) 17 circuli were counted out from the focus, along this axis, (3) one end of a pair of dividers was placed in the center of the focus, (4) the most distant point on the periphery of the seventeenth circulus was determined with the dividers, and (5) the line formed by the last two points was the scale axis. If the seventeenth circulus could not be used, the sixteenth was substituted.

RELATION OF CIRCULUS NUMBER TO DATE OF COLLECTION

Scales of juvenile pink salmon from Puget Sound were examined to determine when the first circulus was formed. Fish were captured April 14, April 20, April 29, May 4, May 11, May 19, and June 23. Only the fish in the sample of June 23 had scales. Two fish, 60 and 63 mm long, had scales without a circulus. Other specimens had scales with one, two, or three circuli. The number of circuli on scales at later dates could not be determined for this area because fish could not be located; scales of juveniles from Kodiak Island and the coast of southeastern Alaska were used instead.

The mean number of circuli on scales of juveniles became progressively larger with later sampling dates (Table I). The mean was 1.7 circuli on the Puget Sound scales of June 23 and 17.4 for fish taken off the coast of southeastern Alaska on September 10. The frequency distributions indicated that the range of circulus number was wide within certain samples (Table II).

TABLE I. Mean numbers of circuli on scales of pink salmon and mean fork lengths, by date of collection.

Locality	Life stage and date of collection	Number of fish	Mean number of circuli	Fork length (*mm*)	
				Mean	Range
		Juvenile			
Puget Sound, Washington	6-23-64	51	1.7	73.8	60–82
Uganik Bay, Kodiak Island	7-9-64	67	2.6	84.8	75–97
No Name Bay, southeastern Alaska	7-13-64	50	8.0	131.9	93–162
Kaigani Point, southeastern Alaska	8-1-64	115	10.7	154.8	135–193
58°12′N, 137°02′W	8-14-64	109	12.7	171.0	135–210
56°39′N, 135°30′W	8-31-64	111	15.3	193.3	135–235
58°32′N, 137°46′W	9-10-64	104	17.4	219.4	140–250
		Adult			
46°30′N, 142°09′W	1-22-64	15	25.4	308.0	245–365
46°30′N, 146°32′W	1-23-64	8	24.1	291.2	225–390
44°55′N, 136°08′W	4-10-63	23	29.7	376.5	335–455
47°05′N, 135°00′W	4-25-65	30	31.5	376.3	330–410
45°50′N, 132°15′W	4-28-63	41	31.3	389.6	345–505
53°17′N, 137°15′W	5-19-64	67	33.7	421.9	360–540
48°00′N, 137°40′W	5-30-65	33	36.3	465.0	390–550
57°23′N, 137°06′W	6-14-63	111	36.9	478.2	400–560
57°00′N, 140°00′W	6-25-65	90	36.8	473.3	415–545
57°37′N, 150°21′W	7-12-63	82	37.2	499.6	430–575
52°20′N, 166°58′W	7-15-62	66	36.4	486.2	429–536
54°01′N, 162°03′W	7-22-63	28	36.1	500.8	466–540
49°52′N, 127°36′W	7-23-65	15	46.3	545.0	460–640
57°18′N, 136°50′W	7-26-63	48	44.0	522.9	470–595
55°30′N, 154°03′W	8-16-62	17	40.5	522.1	477–600
55°32′N, 152°00′W	9-2-62	15	46.9	539.9	488–574

For example, the number of circuli on scales taken August 31 ranged from 9 to 20 and on September 10 from 9 to 22.

Generally, the total number of circuli on scales of adults increased through the collecting season (Table I). The mean number of circuli on scales taken in late January was approximately 25 and on September 2, 46.9. The mean values of the January 23, July 15, July 22, July 26, and August 16 samples were smaller than mean values from earlier samples. A possible reason for the small mean values on at least four of the five dates is discussed in the following section.

The range of circulus number was wide within all scale samples taken from adult fish (Table II). For example, the number of circuli on scales collected January 22 ranged from 18 to 33 and on June 14 from 30 to 46. The narrowest range, 43–50 circuli, was found in the sample taken September 2.

RELATION OF CIRCULUS NUMBER TO FORK LENGTH

The records of mean fork lengths and mean numbers of circuli (Table I) bring out the following two relationships: (1) the mean number of circuli

TABLE II. Numbers of fish listed in Table I with various numbers of circuli on the scales.

Date of collection	Number of circuli on scale																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Juvenile																								
6-23-64	2	18	25	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
7-9-64	–	8	25	23	11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
7-13-64	–	–	–	–	1	–	4	12	16	13	2	2	–	–	–	–	–	–	–	–	–	–	–	
8-1-64	–	–	–	–	–	–	–	–	2	19	29	39	21	2	1	–	2	–	–	–	–	–	–	
8-14-64	–	–	–	–	–	–	–	–	3	9	15	16	13	9	10	18	8	5	1	2	–	–	–	
8-31-64	–	–	–	–	–	–	–	–	–	5	4	1	3	5	8	26	25	20	7	6	1	–	–	
9-10-64	–	–	–	–	–	–	–	–	–	1	1	1	–	3	4	8	8	24	23	16	6	8	1	

	Number of circuli on scale																																			
	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	
Adult																																				
1-22-64	1	–	–	–	1	2	2	3	2	1	1	–	–	–	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
1-23-64	–	1	1	–	2	–	1	–	–	1	1	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
4-10-63	–	–	–	–	–	–	–	1	3	4	5	2	2	2	3	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
4-25-65	–	–	–	–	–	–	–	–	–	–	2	5	3	9	3	1	1	3	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
4-28-63	–	–	–	–	–	–	–	–	2	3	2	6	4	8	2	3	3	3	2	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
5-19-63	–	–	–	–	–	–	–	–	–	1	1	1	4	8	10	7	8	10	6	6	3	1	–	1	–	–	–	–	–	–	–	–	–	–	–	
5-30-65	–	–	–	–	–	–	–	–	–	–	–	1	1	1	1	3	3	2	7	3	2	2	3	2	–	2	–	–	–	–	–	–	–	–	–	
6-14-63	–	–	–	–	–	–	–	–	–	–	–	–	1	–	3	11	10	7	20	14	18	11	5	3	3	1	1	2	1	–	–	–	–	–	–	
6-25-65	–	–	–	–	–	–	–	–	–	–	–	–	1	3	2	5	7	13	12	13	12	5	6	4	6	–	1	–	–	–	–	–	–	–	–	
7-12-63	–	–	–	–	–	–	–	–	–	–	–	–	1	1	2	5	9	9	7	10	8	10	7	7	5	–	1	–	–	–	–	–	–	–	–	
7-15-62	–	–	–	–	–	–	–	–	–	–	–	–	2	–	1	5	8	10	10	13	3	5	4	1	3	–	–	1	–	–	–	–	–	–	–	
7-22-63	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	3	1	5	4	3	5	4	1	–	–	–	–	–	–	–	–	–	–	–	–	
7-23-65	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1	3	4	2	3	–	–	–	1	
7-26-63	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	3	2	6	3	5	8	6	6	2	1	2	3	–	–	
8-16-62	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	1	2	1	1	3	3	2	–	1	1	–	–	–	–	–	–	
9-2-62	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	4	–	3	1	3	2	–	–	

and mean fork length both tended to increase as time progressed and (2) the mean number of circuli generally increased with an increase in mean fork length. Although the ranges in fork lengths for fish with the same number of circuli were wide, mean fork length clearly increased with number of circuli (Table III, Fig. 3). The rate of laying down circuli in relation to increases in length seemed to change at 23 and 33 circuli (Fig. 3). The change at 23 circuli occurred sometime in January or earlier, at about the time of formation of the winter ring. Fewer circuli were laid down per unit change in length between the 23 and 33 circuli than before or after.

TABLE III. Mean fork lengths of pink salmon, in relation to number of circuli on their scales.

Number of fish	Number of circuli	Fork length (*mm*)		Number of fish	Number of circuli	Fork length (*mm*)	
		Mean	Range			Mean	Range
2	0	61.5	60–63	5	26	344.0	320–370
26	1	73.8	65–83	9	27	344.4	285–385
50	2	78.7	70–87	11	28	362.3	335–395
29	3	85.1	76–94	18	29	370.3	335–420
12	4	91.3	86–97	19	30	396.4	350–462
–	5	–	–	34	31	403.1	355–500
4	6	116.0	109–119	28	32	421.9	360–515
12	7	125.2	113–147	47	33	450.9	355–538
21	8	136.2	126–150	51	34	459.2	380–530
47	9	142.9	128–160	63	35	461.9	350–526
51	10	153.2	140–168	72	36	468.8	330–530
59	11	156.2	140–172	66	37	479.7	395–540
37	12	162.9	153–185	54	38	487.8	420–540
19	13	176.7	150–205	42	39	495.6	415–600
23	14	186.3	161–210	29	40	495.0	415–575
52	15	192.4	150–220	27	41	509.2	440–545
43	16	200.6	167–230	24	42	510.0	460–560
49	17	211.7	185–245	11	43	511.3	450–553
32	18	222.5	195–245	13	44	526.5	495–580
25	19	222.6	190–245	17	45	539.8	500–595
8	20	236.9	225–250	12	46	531.1	495–640
8	21	237.5	230–250	7	47	530.9	488–585
4	22	252.5	210–300	5	48	530.2	460–595
2	23	282.5	255–310	5	49	538.4	513–569
3	24	291.7	285–300	5	50	550.6	506–580
3	25	315.0	310–320				

Shorter body lengths may account for the small mean numbers of circuli on scales of adults taken January 23, July 15, July 26, and August 16 (Table I). Fish taken on earlier dates were longer and had larger mean numbers of circuli. Enyutina (1963) also found greater numbers of circuli on longer pink salmon. Fish collected in the Amur River estuary that averaged 367 mm long had 24.9 circuli and fish that averaged 563 mm had 33.3 circuli.

The relation between fork length and number of circuli evidently differed between some stocks. Late in the 1963 season, fish taken off southeastern

Alaska that were approximately 50 cm long had a larger mean number of circuli than fish of comparable length taken east of Kodiak Island and south of the Alaska Peninsula (Table IV). A small number of circuli in relation to fork length was also observed in the sample taken south of Kodiak Island on August 16, 1962 (Table I).

Some information has been published on the body lengths of pink salmon and the number of circuli on their scales. Information available to the author is listed in Table V by dates and areas of collection. Most of the data were compiled from Asian fish. The results of these studies cannot be compared critically in all details for the following reasons: (1) different stocks were examined, (2) scales were taken from different areas of the fish, (3) circuli were counted on different radial lines of the scale, and (4) measurements other than fork lengths may be listed. The mean numbers of circuli and mean fork lengths of Table I and those of Table V merit two comments. First, scale samples from adult pink salmon from the eastern North Pacific Ocean

TABLE IV. Mean numbers of circuli on scales of pink salmon of the same range of fork length (48.5–51.5 cm) from widely separated Gulf of Alaska areas.

Area	Date	Number of fish	Number of circuli		Mean fork length (*cm*)
			Mean	Range	
Off southeastern Alaska	7-26-63	19	43.0	39–46	50.5
East of Kodiak Island	7-12-63	40	36.3	32–41	49.9
South of the Alaska Peninsula	7-22-63	14	36.1	31–39	50.2

appear to have larger mean numbers of circuli than fish of comparable size from the Asian spawning regions of Sakhalin, Kamchatka, and the Amur River. A similar difference was noted by Semko (1939) on the basis of his samples and the two scales collected by Gilbert (1913) in Bellingham, Washington. Second, the body length at the time of scale formation (approx 60 mm) was greater than that observed by Foskett (1951) and Vedenskii (1954). For example, mean fork lengths of fish with two scale circuli were 75.6 mm in Puget Sound and 81.8 in Uganik Bay. The mean body length of pink salmon from Iturup Island with two scale circuli was 44.8 mm in Vedenskii's study, and Foskett showed Vancouver Island pink salmon 52 and 57 mm long with four circuli. The results published by Foskett and Vedenskii were also somewhat different from the findings of Foerster and Pritchard (1943), who noted that scales were formed along the lateral line at $2\frac{1}{4}$ inches (60 mm). Although Neave (1943) did not present data, he also mentioned the " . . . comparatively large size attained by this species (pink salmon) before scale formation begins."

TABLE V. Relation of body length to number of circuli on scales of juvenile and adult pink salmon in Asia and North America.

Dates of collection	Area	Number of fish	Average number of circuli[a]	Average body length[a] (*mm*)	Source
Juvenile					
July 7, August 4, 1946	Iturup Is. (USSR)	212	3 (1–7)	49 (30–80)	Vedenskii, 1954
July 13, 1950	Vancouver Island, B.C.	5	7 (4–10)	76 (52–105)	Foskett, 1951
September 19, 1959	East coast of Kamchatka	2	15	210	Birman, 1960
October–November	West coast of Sakhalin	–	17	230	Dvinin, 1952
Early November 1931	46°31′N, 138°19′E	5	14 (13–16)	– (200–225)[b]	Dubrovskaya, 1934
November 26, 1933	41°28′N, 131°00′E	2	16.5 (16–17)	225.5 (223–228)	do
Adult					
April 1959	Central North Pacific Ocean	47	28	410 (350–460)	Birman, 1960
Late June–July	Western Bering Sea	–	31	480	do
June–July 1948	West coast of Sakhalin	98	32.9	466	Dvinin, 1952
July 1948	East coast of Sakhalin	93	33.4	480	do
July 1929	Amur River (USSR)	10	32.9	546	Semko, 1939
July 1932	East coast of Kamchatka	42	32.9	532	do
July 1934	West coast of Kamchatka	100	28.1	423	do
July 1935	West coast of Kamchatka	98	31.1	472	do
July 1936	West coast of Kamchatka	100	31.6	496	do
August 2, 1910	Bellingham, Washington	1	51	635	Gilbert, 1913
August 3, 1910	Bellingham, Washington	1	50	559	do

[a]Range given in parentheses, where data are available.
[b]Salted specimens.

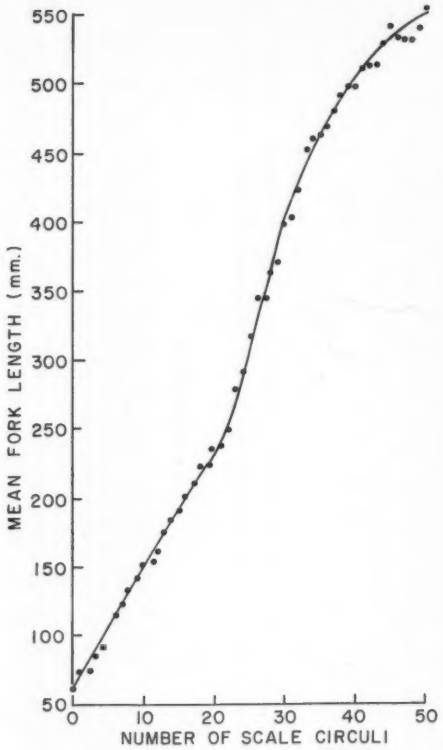

FIG. 3. (*Left*) Mean fork lengths of pink salmon, in relation to number of scale circuli (Table III).
FIG. 4. (*Right*) Scale collected January 23 showing the start of winter ring formation.

TIME OF ANNULUS FORMATION

The winter ring is usually regarded as a winter "mark," formed when pink salmon are growing at a reduced rate. As few pink salmon have been captured on the high seas in winter, little factual information has been published on the time the annulus is formed within the ring. Vedenskii (1954) examined scales of pink salmon from the Kurile Islands and theorized that winter rings were formed in the spring. Birman (1960) examined scales collected in April from four east Kamchatkan fish. He found a winter ring and 7–13 additional circuli. Supported by this scale information and body length data, he stated that the ring was formed" . . . not later than the first half of January." Ricker (1964) used January 15 as the date of annulus formation. This was based on Birman's evidence and scales taken from three fish collected in the Gulf of Alaska, January 24 to February 1, " . . . which had 0, 2, and 2 circuli of new growth."

Scales from both juveniles and adults (Table I) were examined throughout the sampling sequence to detect the presence of annuli and determine dates of their formation. The first winter rings and annuli were found on the scales collected January 22 and January 23; rings were present or being formed (Fig. 4) on 22 of the 23 fish. The appearance of the scales varied considerably. For example, one scale exhibited no evidence of a winter ring while another scale had six circuli beyond the annulus. This variation indicated that the annuli were not being formed at the same time on the scales of all pink salmon. The next sample was taken April 10. All scales of adult fish collected on April 10 and later dates had an annulus.

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Reproductive Rates of Harp Seals, *Pagophilus groenlandicus* (Erxleben)[1]

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ABSTRACT

In less than a decade the mean age at sexual maturity of female harp seals of the Front, or northeastern Newfoundland population, decreased to 4 from 5½ years. Exploitation was heavy during this period and included a high proportion of seals older than 1 year. Females of the Gulf of St. Lawrence herd declined in mean age at sexual maturity over the same decade to 5 from nearly 6 years. Exploitation of this herd was lower, especially for animals older than their first year. Fertility of the adult females was likely higher in the more heavily exploited population. Published data on the White Sea population, which was reduced to low numbers, show reproduction at a lower mean age than for the present Front herd; for the Jan Mayen herd, of uncertain population status, about the same reproductive rate as the present Gulf herd. In most of the populations, samples of adult, whelped females showed the greatest number in the age-class about 1 year older than that first showing 100% of the females mature, as would be expected; however, samples from the Front herd showed dominance of an older age-class, suggesting that young adult females may to some extent segregate into separate groups. The immediate factors leading to increased reproductive rates at lower population densities were not elucidated.

INTRODUCTION

Accurate age determination from growth rings in the canine teeth has allowed determination of ages at sexual maturity of female harp seals, *Pagophilus groenlandicus* (Erxleben), in recent years. The method of age reading is given by Fisher (MS, 1954). Data for the western North Atlantic populations are now available over a period of nearly 15 years. During this time changes in reproductive rates have appeared concurrently with population declines due to increased exploitation. These changes are documented here.

The harp seals of the western North Atlantic are divided into two distinct breeding populations, one of which breeds on the ice of the southern Gulf of St. Lawrence (Gulf population) and the other on the ice of the main Labrador Current in the vicinity of Belle Isle and northward (Front population). Evidence for the separate identities of these populations has been presented by Sergeant (1965).

In these harp seal populations, mating and conception take place in mid to late March with immediate growth of the new corpus luteum. Implantation of the embryo is delayed some 3 months, and birth occurs in early to mid March of the following year. The basic reproductive cycle has been discussed by Fisher (MS, 1954), whose data have been used in this study, and indeed form its baseline.

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MATERIALS AND METHODS

The samples consisted of ovary pairs from females together with a lower canine tooth sectioned for determination of age from growth layers.

The Gulf population was sampled from migrants entering the Gulf in early January. At this time the pregnant females carried large foetuses conceived the previous March, so that the age at conception was referred back to that date. For the Front population, sampling of moulting animals was carried out from late March to late April. By this time conception had taken place and the new corpus luteum was developing, while the corpus luteum resulting from the birth in early March was little regressed and could be separated by its size and lutein content from the smaller, fibrous scars of earlier pregnancies. Thus the moulting females gave evidence of pregnancy in both the current and the immediately past season of reproduction. The age of these animals was expressed as the age attained in March a month or two previous to sampling.

Young females with regressing corpora lutea, several of which were found in the January samples from the Gulf, were included among mature females in order to be comparable with the Front females. In the latter, a developing corpus luteum was necessarily considered the index of maturity, even though it might later regress. Thus age at maturity as here defined is age at first ovulation, not first conception.

Age reading of Gulf samples was carried out by Fisher from collections made in 1951–54 and by the author from collections made in 1964 and 1965. Front samples were collected and read by Fisher in 1953 and by the author from 1952 to 1954, and in 1962. Since we worked together and compared readings of many harp seal teeth over a number of years there should be no important discrepancy in age readings of younger animals, and inevitable discrepancies for older animals are not important in the present context.

MEAN AGE AT SEXUAL MATURITY

Tables I and II show the information obtained from the different samples which numbered from 50 to 250 animals. Figure 1 summarizes the results.

On the Front, samples by Fisher and by the author both with a mean date of 1953 showed similar results (Table I). Sexual maturity was attained at 4–7 years with a mean age of about $5\frac{1}{2}$ years. These samples showed no 3-year-olds and only one 4-year-old to be mature.

The author's scanty data for 1962 were increased by a sample taken at the Front in 1961 from a Soviet icebreaker by R. Sh. Khuzin, who published full data on sexual condition of 39 females aged 1–7 years (Khuzin 1963, table 4). An analysis of Khuzin's data is included in Table I. It shows that the 1961 and 1962 data were in agreement, and contrasted sharply with those of 1952–54. In the later samples there were no immatures older than 5 years, and the mean age at maturity was close to 4 years. Tests of significance were made on these data using a 2×2 contingency test for the ages showing greatest actual or expected divergence. Comparing data for 1952–54 with those for

TABLE I. Reproductive states of female harp seals from the Front for two periods, showing (bars) ranges in age at first maturity and (horizontal lines) mean ages when maturity was attained. Samples taken from moulting seals in April.

Age (*years*)	1953 (Fisher) Total	Mature	(Infertile)	1952–54 (Author) Total	Mature	1962 (Author) Total	Mature	1961 (Khuzin, 1963) Total	Mature
1	4			2		8		7	
2	1			4		10		2	
3	12			6		[6	1]	2	
4	13			[3	1]	[8	4]	[6	3]
5	[17	6]		[7	2]	[5	3]	[5	3]
6	[6	4]		[9	8]	2	2	7	7
7	3	3		[8	7]	1	1	10	10
8	2	2	(1)	6	6				
9	4	4		5	5	2	2	*No data*	
10	2	2	(1)	4	4				
11	2	2		6	6	1	1		
12	2	2		3	3				
13	2	2		1	1				
14				1	1	2	2		
15	1	1		4	4	2	2		
18				3	3	1	1		
19									
20									
21				1	1	1	1		
22						1	1		
23									
24	1	1							
25				1	1				
10+	1	1							
20+	1	1							
Total:	74	31	(2)	74	53	50	21	39	23

1961 and 1962, age-group 4 exhibited a greater divergence ($P = 0.0125$) than age 5 ($P = 0.15$) and the first value is highly significant.

In the Gulf (Table II), samples were collected by Fisher in 1951–54 from the net fishery at La Tabatière. Sampling was again carried out in 1964, this time from the net fishery at Harrington Harbour a few miles west, and in 1965 a large sample was obtained from both Harrington Harbour and La Tabatière. Results for the two localities, and for the two years 1964 and 1965, were in good agreement. Between the two periods, the mean age declined from approximately 5½ years to just under 5 years. Tests of significance for ages 4 and 5 years did not give highly significant results, evidently because numbers were small for these ages in the early sample. However, the decline had not proceeded so far as on the Front. Moreover in the Gulf there were still immatures up to an age of 7 years.

In summary, mean age at sexual maturity on the Front declined in less than a decade by about 1½ years, from 5½ to 4 years. In the Gulf it declined over a decade by less than 1 year, from about 5½ to 5 years.

TABLE II. Reproductive states of female seals from the Gulf of St. Lawrence for two periods. Symbols as in Table I. Samples taken from southward migrants in January of each year.

Age (*years*)	1951–54 (Fisher)			1964 (Author)			1965 (Author)		
	Total	Mature	(Infertile)	Total	Mature	(Infertile)	Total	Mature	(Infertile)
1	0			8			5		
2	1			16			17		
3	5			11			25	2	
4	4			9	2		34	4	
5	3	1		2	1		33	21	
6	3	2		4	3		34	27	
7	16	13		5	5		32	31	
8	4	4		4	4		14	14	(2)
9	8	8	(1)	2	2	(1)	5	5	
10	8	8	(1)	1	1		4	4	
11	4	4		6	6	(1)	9	9	
12	3	3		1	1		8	8	(2)
13				1	1		7	7	
14	2	2					6	6	
15				1	1		2	2	
16	2	2	(1)	2	2	(1)	4	4	(2)
17									
18				1	1		6	6	(1)
19							1	1	
20	3	3	(1)						
20+	3	3	(1)	1	1	(1)	3	3	
Unaged	1	1	(–)[a]						
Total:	70	54	(5)	75	31	(4)	249	154	(7)

[a]Resorbed embryo.

Age samples of the adult females may be collected from kills of animals guarding young at the whelping patches, and such samples have now been collected by various authors from all four populations of harp seals. Ignoring fluctuations in strength of a particular age-class from year to year, such samples should show as the dominant age-class that in which for the first time all females are whelping, i.e. an age 1 year greater than that at which all females have become mature. A sample of 113 breeding females collected by the author in the Gulf in 1954 (Fig. 2A) showed dominance of the 10-year-old females. From Table II, in 1953, 9-year-olds should be dominant. Considering the small size of the reproductive sample, agreement is good.

At the Front, Soviet scientists have collected age samples of adult, whelped females. Khuzin (1963, table 2; data smoothed in my Fig. 2B) found 11-year-olds dominant in a sample collected in 1961. Popov and Timoshenko (1965) found 9-year-old females dominant in a sample collected in 1963. Both ages were somewhat higher than would be expected from reproductive samples of this date (Table II) which indicate that all females were giving birth at 6 or 7 years. It is possible, as has long been claimed by Newfoundland sealing men, that young females whelping for the first time segregate to some extent into

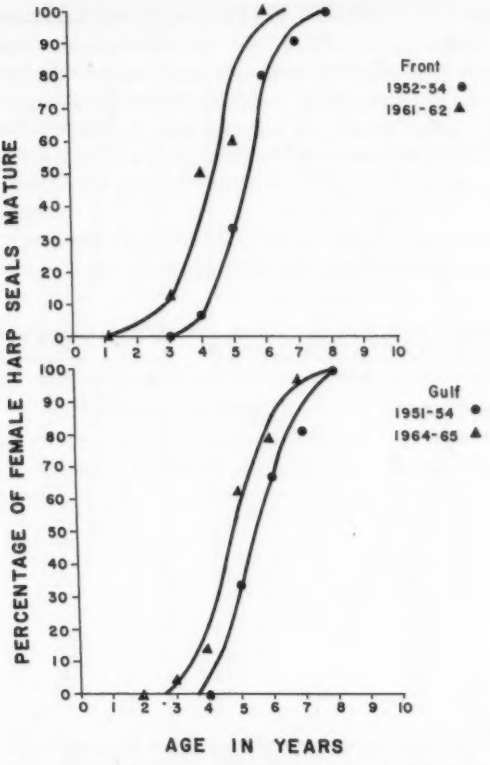

FIG. 1. Changes in age at maturity of female harp seals. Data from Tables I and II.

separate groups. No other population shows this discrepancy. However, the dominant age-class in these samples will be influenced within small limits by differential survival of particular year-classes.

FERTILITY OF OLDER FEMALES

On the Front, Fisher's 1953 data (Table I) showed 2/18 or 11% of adults 8 years and up to be infertile in the current year, while the author in 1952–54 did not have full data on this question. In the Front sample of 1962, of 10 females older than 8 years, all were pregnant in the current year, one having twin corpora lutea, and all had been pregnant in the previous year as judged from the size of the last corpus albicans.

In the Gulf in 1951–54, of females 8 years old and older, 5/37 or 14% were without a corpus luteum of the current year (Table II). In 1964, 4/25 or 16% were infertile, and in 1965, 7/69 or 10%. The mean for the Gulf was 12%.

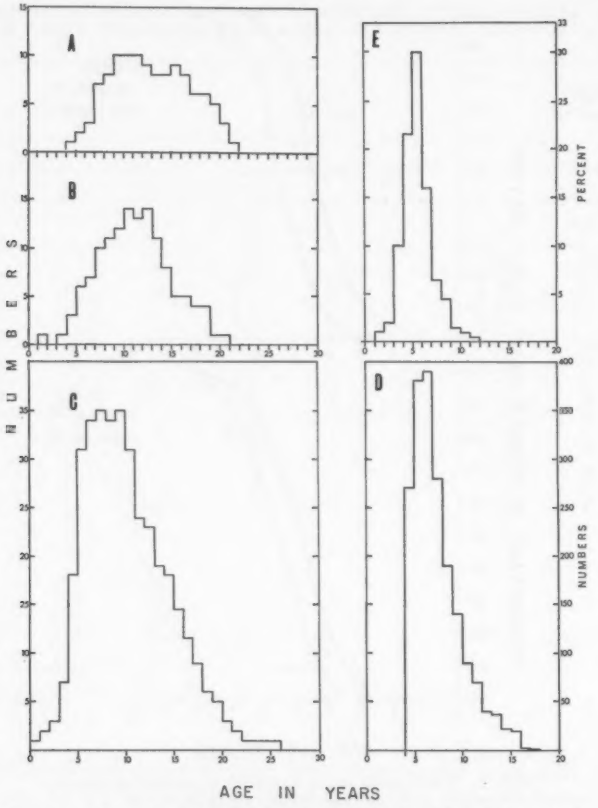

FIG. 2. Selected age frequencies of newly whelped female harp seals from the four populations. All curves smoothed by 3s except for D. A — Gulf of St. Lawrence, 113 animals, 1954 (present paper). B — Front, 127 animals, 1961 (Khuzin, 1963). C — West Ice, 370 animals, 1963 (Khuzin and Potelov, 1963). D — White Sea, 1959 animals, 1962 (Khuzin, 1964). E — White Sea, 1234 animals, 1961 (Nazarenko and Yablokov, 1962).

Front females thus showed marginally higher fertility rates than Gulf females ($P = 0.126$), and there was a suggestion that the level of fertility had risen over the 10-year period on the Front but had not measurably changed in the Gulf.

DISCUSSION

The two populations of seals have been estimated on several occasions by aerial photographic census of breeding adults and young (Sergeant and Fisher, 1960). Computations based on life tables are necessary to convert these figures to total population, so that only the figures of young born are

given here. These are: for the Front, in 1950 — 430,000, and in 1960 — 215,000. For the Gulf, equivalent estimates are in 1950 — 215,000, and in 1960 — 150,000. There was no change from this last estimate in a survey of the Gulf made in 1964. The aerial surveys are subject to large errors and are probably in many cases underestimates of the number of young born. Nevertheless, the results taken together indicate that numbers have declined considerably on the Front, much less so if at all in the Gulf. This is the result of much heavier hunting on the Front.

Catches of harp seals in the Gulf and on the Front (Table III) showed that the kill of young on the Front in 1950–59 was about twice that in the Gulf,

TABLE III. Catches of harp seals in the western North Atlantic from 1950 to 1964, in thousands of pelts.

	Gulf of St. Lawrence		East of Newfoundland (Front)	
Year	Young	Immature and adult	Young	Immature and adult
1950	31	14	142	28+[a]
1951	90	16	146	52+
1952	63	15	250	77+
1953	32	–	148	62+
1954	74	2	114	62
1955	94	8	160	67
1956	93	14	249	34
1957	74	4	80	75
1958	90	11	60	135
1959	62	7	180	74
Average: 1950–59			1954–59	
	70	9	154	77
1960	84	16	93	112
1961	43	6	133	12
1962	90	16	163	97
1963	95	21	197	64
1964	81	17	178	63
Average: 1960–64	79	15	153	70

[a]Landsmen's catch unknown, 1950–53.

which is proportional to the magnitudes of estimated populations in 1950. However, kills of adult and immature seals in 1954–59 were higher on the Front than in the Gulf by a factor not of 2:1 but of nearly 9:1. Since 1962, there has been increasing control of this catch in both areas by a closing date for sealing.

There has been some increase in catch of both young and older seals in the Gulf in recent years (Table III), and it is supposed that much of the decline in reproductive age here has occurred recently. This conclusion is supported by the observation that there are still immature animals in the Gulf herd up to an age of 7 years.

Recent studies, although not fully documented, give the mean age at sexual maturity of female harp seals from the other two world populations of the species. For the White Sea, Chapsky (1963, p. 228) gives the following summary for collections of females apparently made in 1946–48: 30% mature at age 3, 83% mature at age 4, 100% mature at age 5. Chapsky makes clear that the age given is that at conception, with birth a year later. This is an even younger mean age at maturity than for the Front females. It is confirmed (Fig. 2E) by samples of females which from absence of immatures were evidently mainly breeding females, collected in 1961 by Nazarenko and Yablokov (1962). These show maximum frequency of females at 6 years, which would correspond to full sexual maturity at 5 years as found by Chapsky. Khuzin (1964, table 4; my Fig. 2D), found 6- and 7-year-old females codominant among adults in the White Sea in 1962.

The White Sea population of harp seals was greatly reduced in numbers during the 1930s, the total population as estimated from aerial survey in 1952–53 being about 1,250,000–1,500,000 (Surkov, 1957) as compared with 3,000,000 in 1928 (Dorofeev and Freimann, 1928). Subsequently, it was reduced to some 750,000 (Nazarenko and Yablokov, 1962). These facts suggest that the mean age at sexual maturity of female harp seals in the White Sea in 1946–48, the time of Chapsky's sampling, approached the maximum reproductive potential of the species.

For the Jan Mayen or West Ice harp seals, studied in 1962, Khuzin and Potelov (1963, p. 49) found immature females up to an extreme age of 6 years, with first maturity inferred from 3 years. This is confirmed by a sample of females (their table 2 and figure 2), most of which appear to be whelping animals because few immatures are represented. These figures (smoothed in my Fig. 2C) show females increasing in frequency stepwise up to 8 years of age. These data give a mean age at sexual maturity of about 5 years, about the same as the present Gulf population, but higher than White Sea and present Front populations.

The population status of the West Ice herd of harp seals is not well known. Surkov (1957) considered the stock to be in fair condition, basing his conclusions on relatively low recent catches and the numerous old animals present in samples as compared with the White Sea stock. Rasmussen (1957) shows that West Ice catches have been chiefly of younger animals. In this catching field hood seals, *Cystophora cristata* Erxleben, are numerous and much hunting is concentrated on them.

Concerning other species of seals, Carrick and Ingham (1962) showed that females of a little-exploited population of southern elephant seals, *Mirounga leonina* L., at Macquarie and Heard Islands showed a mean age at sexual maturity of a little over 3 years. In contrast, females of a heavily-exploited population at South Georgia had been shown by Laws (1956, p. 21) to have a mean age at sexual maturity of just over 2 years, evidently the maximum reproductive rate for the species.

Chapman (1965, table 5) gives pregnancy rates of female North Pacific fur seals, *Callorhinus ursinus* (L.), at North American and Asian colonies, and shows that Asian fur seals first attain a high rate of pregnancy (about 0.80) at 5 years, North American seals at 6 years. This difference is again related to a difference in population status; North American (Pribilof Islands) colonies have long ceased to grow in numbers, whereas Asian (Commander Islands) colonies are still actively growing.

The factors inducing lower reproductive rates at higher population densities are as yet unknown. It is possible that in a population approaching its natural maximum, competition for food between the young seals leads to delayed growth and maturity. In this case, mean weight at a given age in the population near its maximum should be lower than mean weight at the same age in the reduced population; as Laws (1959) showed, accelerated growth in seals is positively correlated with earlier sexual maturity, a conclusion which should be valid within a species as well as between species. However, weighings of harp seals of known age were not carried out in this study.

It is also possible that the older seals compete with the young for food, although there is some seasonal segregation in food selected; the young seals feed principally on euphausiids, the immatures on capelin, *Mallotus villosus* (Müller), the adults on larger benthic fishes such as cod and flatfish. If such competition should occur, a certain degree of thinning of the adults may have some virtue in management. It has been noted above that the kill of young seals in the Gulf and Front herds has been in proportion to population, while the kill of older seals has been much heavier on the Front, and it is the Front seals which have shown the greater lowering of age at maturity.

At any rate, it is clear that in an approach to maximal population in seals, sexual maturity is progressively delayed in many animals, and probably, the fertility of older females is reduced. One may suppose that the population is limited also by increased natural mortality, especially of the young.

Lastly, the different reproductive rates of the Gulf and Front populations in recent years give further evidence of their separate identities.

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NOTES

A Simple Technique for Obtaining Chromosomes from Teleost Fishes[1]

Chromosomes have been used extensively in taxonomic work in the invertebrates, but technical difficulties have limited their use within the vertebrates. In fishes, several methods have been employed. The most successful of these have been tissue cultures (Roberts, 1964), colchicine injections and squashes of the testis or haemopoetic tissues (Roberts, 1964; Ohno et al., 1965), squashes of embryological material (Simon, 1963; Simon and Dollar, 1963; Swarp, 1959), or sectioning of testis (Nogusa, 1960).

All of these methods have given usable results, but are time consuming and often yield low percentages of countable chromosomes. The following technique yields a high proportion of countable chromosome sets from a single preparation in a very short time, and has the virtue of leaving the specimen intact for further taxonomic studies.

At present the technique is empirical and may have to be modified for some groups of fishes. It is most successful on freshwater fishes, and has given usable results in several marine groups. The procedure given below is for juvenile fishes 2–6 inches in standard length. For larger fish both the colchicine dose and the length of time after injection should be increased.

Procedure:

1. Make an intermuscular injection of 0.1 ml of 0.01–0.05% (depending on the size of fish) colchicine or Velban in the anterior portion of the back.
2. Place the injected specimen in a well-aerated container for 1–2 hr.
3. Kill the specimen and remove the posterior gill arch.
4. Dialyze the arch for 30 min in distilled water.
5. Stain for 15 min in fresh, *filtered* aceto-oricine.
6. Shake the stained arch lightly on a *clean* slide until a light slurry of cells is deposited on the slide. Remove any large pieces of tissue from the slide.
7. Cover immediately with a clean cover glass, place the preparation on a clean, flat surface (i.e. a piece of plate glass or thick plexiglass) and squash manually using a number 5 or 6 rubber stopper.

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8. The preparation is now ready for examination, or can be sealed with lanolin-paraffin for future study. If stored in a refrigerator, sealed preparations will last for several days.

This technique appears to be most successful on young, actively growing fish in good condition. In marine fishes, the best results were obtained by using higher doses of colchicine (around 0.1 ml of 0.5% colchicine) and leaving the fish for at least 12 hr before preparing the tissue. In addition, placing the fish in 50% sea water for 1 hr, and then in fresh water for another hour before removing the gill arch, seems to improve chromosome spread in most marine species.

Other epithelial cells, especially from the paired fins, are sometimes usable, but in general gill epithelium has consistently given the best results. Figures 1 and 2 show chromosomes obtained from a freshwater and marine fish using this technique.

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(Fig. 1 and 2 *opposite*)

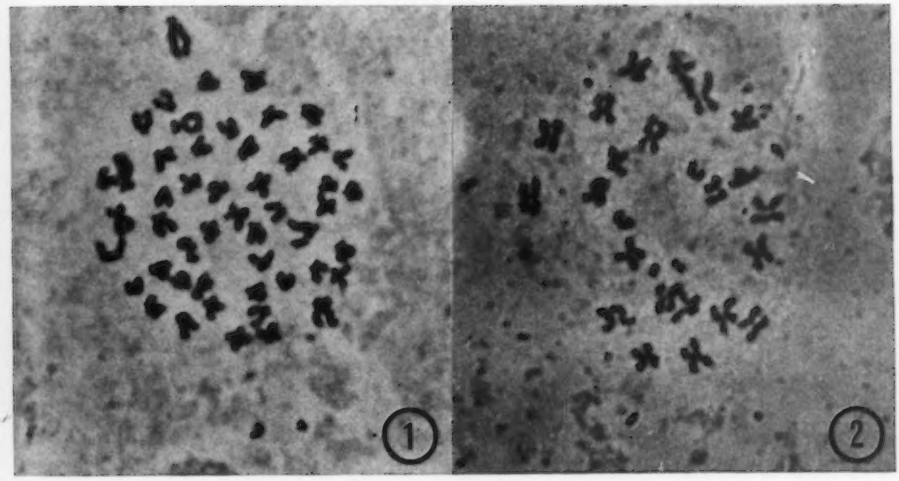

FIG. 1. Metaphase chromosomes from gill epithelium of *Rhinichthys evermanni*. × 2000. 2N = 50

FIG. 2. Metaphase chromosomes from gill epithelium of *Ronquilus jordani*. × 1800. 2N = 26

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Analysis of an Aerial Census of Ringed Seals

Local abundance of ringed seals (*Phoca hispida* Schreber) depends on availability of fast ice suitable for reproduction. Population estimates have been made from counts of seals basking on the ice in spring, together with measurements of ice areas on maps of the eastern Canadian Arctic (McLaren, 1958, 1961). Estimates were based largely on a series of counts from Ney Harbour, a small fiord in Frobisher Bay, in 1957. Counts made there in 1962 were similar. Seals were scarcer during bad weather (which predominated in late June of both years), but the envelope-curve in Fig. 1 describes numbers

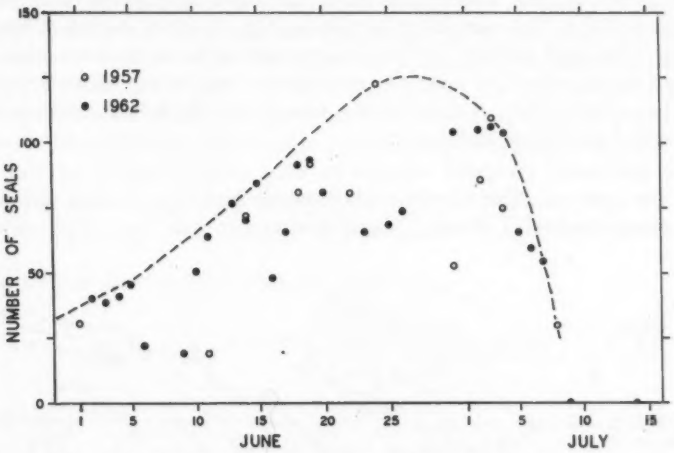

FIG. 1. Numbers of seals counted on the ice of Ney Harbour.

basking during good weather. There was a marked cutback of ice and increase of seal *density* in late June of 1962, but this did not appear to affect *numbers* compared with those of 1957. All age-classes are on the ice at the peak of basking, and the maximum number (123) can be taken as a safe estimate of the population. This reflects a density of almost 50 seals per square nautical mile of the original ice area (2.6 square miles) of the Ney Harbour. The value of 35 seals per square mile used previously (McLaren, 1958, 1961) was low due to overestimation of the ice area.

For estimating populations elsewhere it was previously assumed that the density of seals in Ney Harbour could be applied to all ice within 1 mile of land but greater than 1 mile from the seaward edge of the fast ice. Because the ringed seal has become more valuable and more heavily exploited recently, it was thought that aerial census of a number of localities around southeast Baffin Island would give safer estimates.

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Censuses were made at the height of the basking season on June 27–28, and July 2, 5, and 8, 1964, by Mr Brian Beck of the Fisheries Research Board's Arctic Biological Station, assisted by Miss Elizabeth McClintock of the Department of Northern Affairs and National Resources. It was found that seals were scared down by the aircraft, so each census was made by a single flight at low level (ca. 100 ft), usually down the middle of prechosen bays and fiords. The observers, one on each side, attempted to count all seals to the limit of visibility. The weather was warm and sunny or slightly overcast, except on July 2, when a flight was cut short by cloud and freezing.

Twenty localities were censused. Three of these were partly or entirely pack ice at the beginning of the series. Three other localities had seals when first censused, but had no fast ice on subsequent visits. These six localities were excluded on the assumption that the peak of basking was past even on the first visits. The remaining localities (Fig. 2) were visited at least twice and were composed entirely of fast ice and had seals on each occasion (except L_1, which consisted of ice near the mouth of a bay which was partly broken up on the second visit; a comparable area of ice slightly further in the bay was censused on the second visit).

The maximum recorded number in each locality (given on Fig. 2) was used in the analysis. The relationship between numbers of seals and ice areas gives a mean density of about 7.5 seals per square mile, not at all comparable

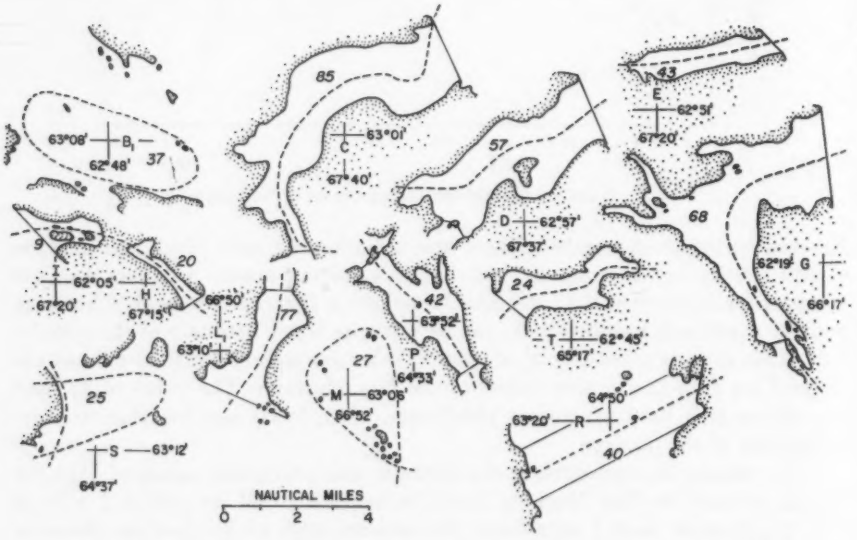

FIG. 2. Localities censused from aircraft in southeast Baffin Island, 1964. Observation was from both sides of the aircraft except in localities B_1, M, and S, which were covered from the inward side. Shorelines and light lines delimit censused ice. Broken lines are aircraft flight paths. Slanted figures are maximum counts of seals.

with the peak density of almost 40 per square mile observed from land on Ney Harbour.

Observed numbers are plotted against calculated numbers (from mean density and ice areas) in Fig. 3a. There is a tendency for larger areas to have fewer and smaller areas to have more seals than calculated. In the larger areas, seals may be scarcer on offshore ice and distant seals may not have been as readily seen. Thus, a complete analysis should integrate functions of distance from shore and aircraft of every point visible from the aircraft. There is no way of treating such integrals analytically, but an approximation may be made graphically by assuming that seals are proportionately less likely to be seen with distance from the aircraft and proportionately less common with distance from shore.

The ice areas on Fig. 2 were enlarged severalfold on graph paper, and the estimated flight path of the aircraft was drawn in by the observer, Mr Beck. Then categories of ice were delimited at 0.2-mile intervals from the shore and from the aircraft's flight path. Areas of fast ice (A) in joint categories of distance from shore and distance from aircraft were estimated by counting squares. Relative density (d) of seals on each joint category of ice was taken as

$$d = 1 - \frac{\text{distance from shore in miles}}{C_1 \text{ in miles}},$$

and proportion observed (p) from the aircraft as

$$p = 1 - \frac{\text{distance from aircraft in miles}}{C_2 \text{ in miles}},$$

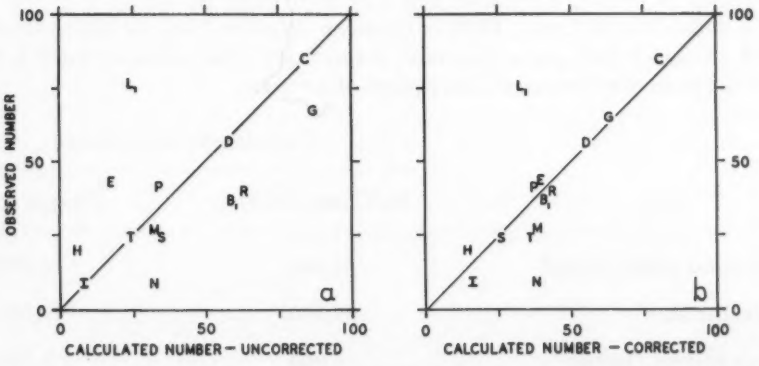

FIG. 3. Observed numbers of seals and calculated numbers (diagonal is line of identity) on the ice of each locality in Fig. 2. Fig. 3a shows relationship assuming proportionality between area censused and numbers of seals. Fig. 3b shows relationship based on the assumption that there is a proportionate decrease of numbers with distance from shore and that none occurred beyond 1.1 miles, and a proportionate decrease of visibility with distance from aircraft and that none were seen beyond 0.7 mile. This combination gives smallest sum of squares of deviations of observed from calculated numbers.

where C_1 and C_2 are constants for all localities. The value of $\Sigma A \cdot d \cdot p$. was derived as an index of the *relative* number of seals which should have been seen in each locality for different values of C_1 and C_2. The indices for all localities were summed, the sum was then divided into the total number of seals (562) observed in all localities, and the quotient multiplied by the index for each locality to give a calculated number of seals for that locality. By changing C_1 and C_2 independently, successive approximation was made to that combination of values giving smallest sum of squares of deviations of observed from calculated numbers. These values are 1.1 and 0.7 miles, respectively, and lead to the corrected relationship of Fig. 3b.

Ney Harbour (E on Fig. 3b) is fairly representative. Yet, from the land-based counts of 1957 and 1962 it can be calculated using the above distance-functions that 88 (rather than 43) seals should have been seen from the aircraft. It seems clear that some seals were being missed, and in fact some were observed being scared down by the aircraft. If the same proportion was missed on all classes of ice, then the above value of C_1 would still apply. Then land-based counts and areas of ice in different categories on Ney Harbour can be used to calculate absolute density of seals as a function of distance from shore. This function is:

$$\text{Density per square mile} = 57\text{–}57 \frac{\text{distance from shore in miles}}{1.1 \text{ miles}}.$$

This formula has been used to recalculate seal numbers in southwest Baffin Island, Frobisher Bay, and northwestern Quebec (areas 8, 11, and 15, in McLaren, 1958). Maps of these coasts were enlarged several-fold photographically. It did not seem practical to delimit more than three distance categories of 0–0.37, 0.37–0.74, and 0.74–1.10 miles (excluding exposed fast ice as defined in McLaren, 1958), with mean densities from the above formula of 48, 28, and 9 seals per square mile, respectively. The following table is constructed from planimetric measurements of ice areas.

Area	Calculated populations	
	McLaren (1958)	Present study
Southwest Baffin Island	61,000	70,800
Frobisher Bay	52,700	47,200
Northwestern Quebec	5,000	3,500

Thus, the results do not differ markedly and, as might be expected, the numbers are increased on the complex coast, with the greatest amount of ice near shore, and decreased on the simplest coast.

It does not appear worthwhile on the basis of this rather over-elaborate analysis to attempt to correct estimates of seal numbers given by McLaren (1958). High-altitude aerial photographs might be necessary to obtain an accurate relationship between numbers of seals and distance from shore. Even then, all seals may not be basking on the ice. More shipboard censuses (McLaren, 1961), and perhaps complete counts of pupping lairs on selected areas of ice, might provide better estimates of absolute density of seals.

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Some Equilibrium Moisture Values of Fresh and Salt Cod

The purpose of this communication is to record a series of determinations of the relationship between the equilibrium water content and relative humidity for salt cod of various degrees of salt content. Such determinations of the "equilibrium moisture content" are a necessary preliminary to any analytical treatment of the air-drying of salt fish.

A limited number of determinations on salt cod muscle have been made previously, but the range of salt concentrations encountered in commercial practice has not been covered. Cooper (1938) described the equilibrium moisture content of "heavy" salted cod. Dingle (1952) gives results for salt cod averaging 27.4% salt (dry basis). Cutting et al. (1956) give a comprehensive review of these and other determinations, and Jason (1958) preceded an exhaustive analysis of the air-drying of fresh cod muscle by a determination of its equilibrium moisture content at 30 C (86 F) and various relative humidities.

Moisture equilibrium weights were measured on a modified McBain-Baker absorption balance using beryllium-copper springs in place of the more usual quartz spirals. In practice the calibrated springs showed no hysteresis and maintained their straight line calibration over a period of years.

Samples were suspended from the springs, the equipment evacuated, moisture admitted to give the desired relative humidity, and the amount of absorbed water determined by measurement of the spring length. Original weight and dry weight at 0% relative humidity could be read in the same manner. That temperatures in the range 20–35 C (68–77 F) had no effect on equilibrium water values (Cooper, 1938) was confirmed by a set of preliminary experiments. In consequence, all observations were made at 20 C (68 F). The control of relative humidity by the addition of water to the evacuated equipment had the advantage of simplicity but in practice the absence of a source of constant vapor pressure, unaffected by small amounts absorbed, caused a hunting between the three suspended samples. This obscured any hysteresis between the wet to dry direction and the reverse (Cooper, 1938) which might otherwise have been observed.

Post rigor cod fillets were salted in the usual manner. Samples averaging about 4.0 g, cut from the prepared fish, were used for determinations as described above. An immediately adjacent sample was analyzed for salt (Anon. 1960. A.O.A.C. method No. 18.008).

The spring length could be read to about 0.02 mm or approximately 0.001 g. Relative humidities were calculated from the readings taken on a mercury manometer to the nearest 0.03 mm. High relative humidities could thus be determined with a precision of about 0.1%; lower values to approximately 3%. An average precision of the equilibrium water content in mid–high range was of the order of 0.5%.

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The results on cod muscle are shown in Fig. 1 and 2. Figure 1 is in the usual form in which per cent equilibrium water (dry basis) is shown as a function of the relative humidity for concentrations ranging from 14.1% salt to 48.6% (dry basis).

If the weight of contained salt is subtracted from the total dry sample weight leaving presumably the weight of dried fish muscle, and if the latter be used as a base for the calculation of the moisture regain, then all values of equilibrium moisture content fall on the single curve shown in Fig. 2.

The curve of moisture regain for fresh cod muscle is shown for comparison on Fig. 1 and repeated in Fig. 2.

Figure 3 shows the equilibrium moisture content for fresh cod skins. The equilibrium curves agree with those determined by Cooper (1938) for heavy, salted fish, but differ considerably, particularly at low relative humidities, from the curve obtained by Dingle (1952) at 27.3% salt (dry basis). The equilibrium moisture curve for fresh muscle lies somewhat below that obtained by Jason (1958) at low relative humidities but agrees within the limits of error in the high range. Agreement with the moistening cycle of the equilibrium moisture content of vacuum freeze-dry raw cod as reported by Cutting et al. (1956) is within the limit of error.

The reasons for disagreement are not clear. It is probable that with the larger samples generally used in obtaining equilibrium values in air over

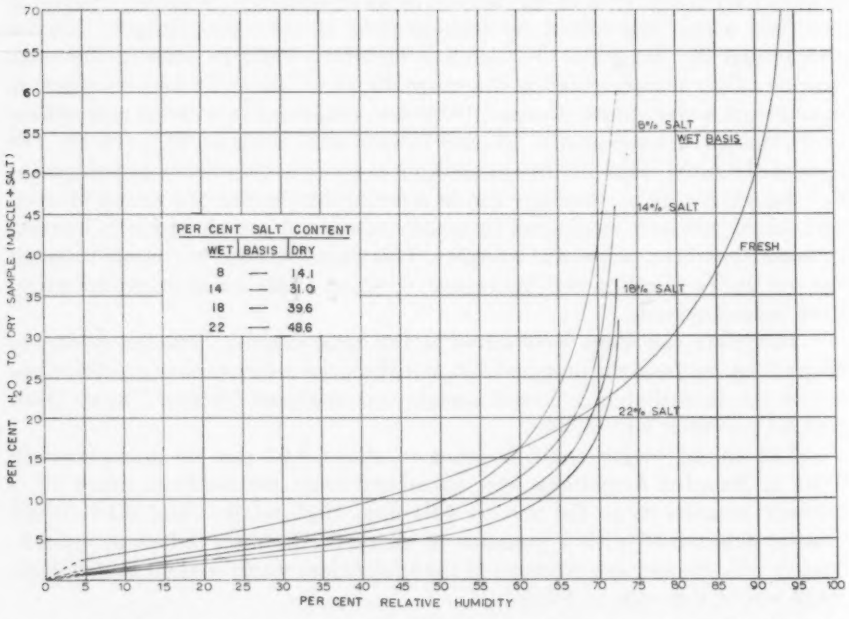

FIG. 1. Equilibrium water content of salt and fresh cod fish as per cent dry weight of sample.

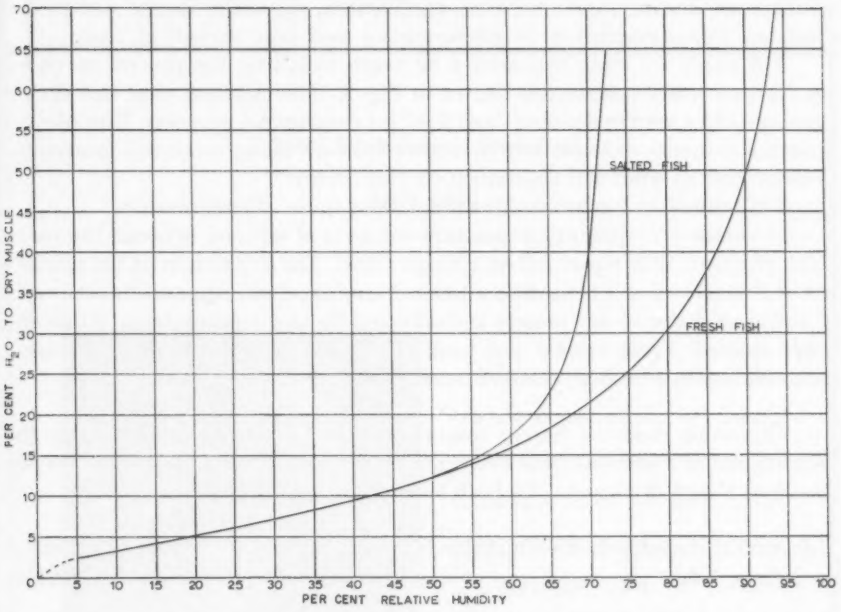

FIG. 2. Equilibrium water content of salt and fresh cod as per cent dry weight of the sample less the contained salt.

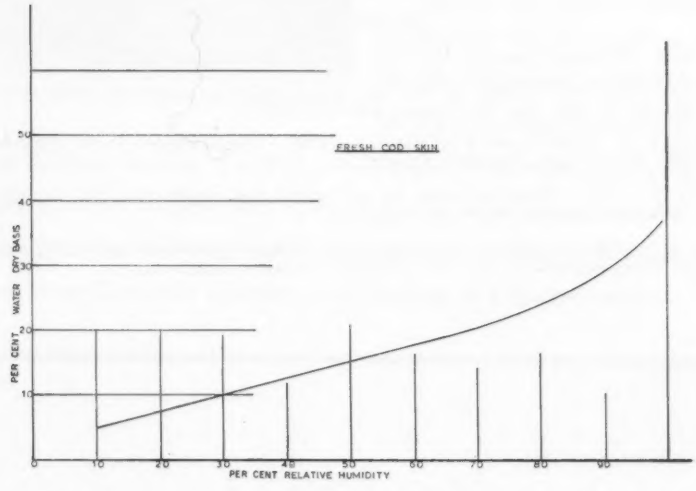

FIG. 3. Equilibrium water content of fresh cod skins as per cent dry weight of sample.

solutions of known vapor pressure, equilibrium, especially in the wet to dry direction, is not reached or is indeterminate over long periods of time.

The curve for fresh fish cannot be made to fall on the general curve for equilibrium water contents as shown in Fig. 2. The criterion that this should occur might be used in defining "salt fish" for commercial purposes. Equilibrium moisture contents at lower salt concentrations are being measured to attempt to determine at what salt concentration this occurs.

The values for fresh cod skins differ from those of fresh muscle.

In summary, equilibrium moisture contents of salt cod between the range of 14–48% salt (dry basis) fall on a single curve. The dry weight of the samples less the weight of salt is used as a basis of computation. Apparently, the ratio of absorbed water to dry muscle is unaffected by salt concentration within the range studied. Fresh muscle and fresh skin tissue do not absorb in the same ratio, particularly at high relative humidities.

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John Richardson Dymond

1887-1965

Ashley & Crippen, Toronto

With deep regret, the Fisheries Research Board of Canada notes the passing of one of Canada's most eminent biologists, John Richardson Dymond. Dr Dymond died in Toronto on Sunday, January 31, 1965, at the age of 77.

This Journal was privileged to honour Dr Dymond in its March 1963 issue in appreciation of his important contributions to fisheries science. In that issue, grateful acknowledgment was made of his twenty years of distinguished service as a member of the Fisheries Research Board of Canada and his significant influence on many Canadian scientists now engaged in fisheries research.

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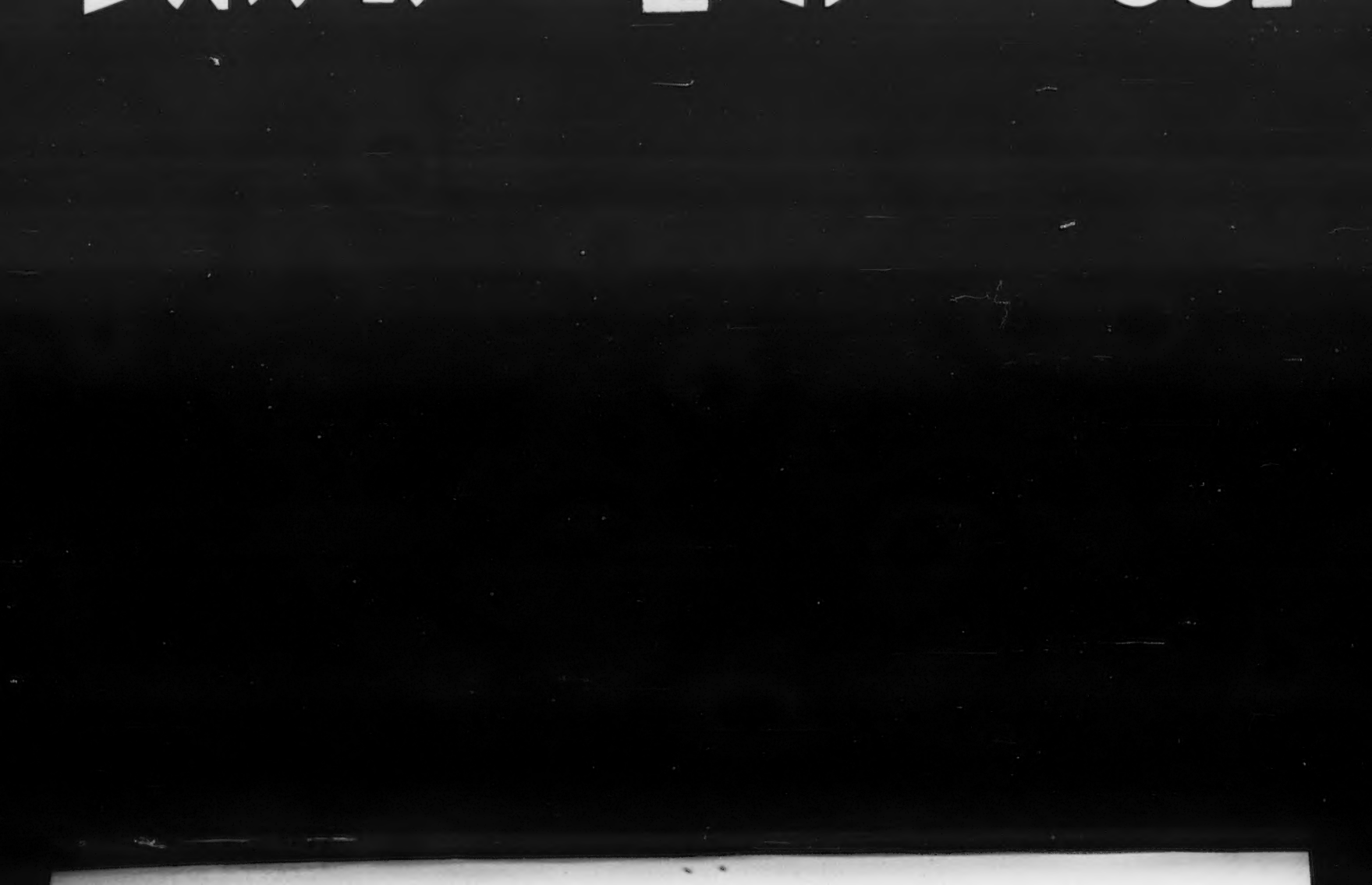
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